1871288CD1_PRT_17_PF-0637-PCT 252 aa

TMEPAI 287 aa

Transmembrane prostate androgen induced RNA, binds the WW domains of NEDD4 ubiquitin-protein ligase, expression is stimulated by TGFbeta, EGF, and other growth factors, several isoforms exist, altered expression is associated with numerous types of cancer

Match: Length=252, Identity: 99%, Similarity:99%, Query Overlap: 100%, Subject Overlap: 87%, E-value:1e-152, Score:520

```
Query: 1   MAELEFVQIIIIVVVMMVMVVVITCLLSHYKLSARSFISRHSQGRRREDALSSEGCLWPS 60
           + ELEFVQIIIIVVVMMVMVVVITCLLSHYKLSARSFISRHSQGRRREDALSSEGCLWPS
Sbjct: 36  ITELEFVQIIIIVVVMMVMVVVITCLLSHYKLSARSFISRHSQGRRREDALSSEGCLWPS 95

Query: 61  ESTVSGNGIPEPQVYAPPRPTDRLAVPPFAQRERFHRFQPTYPYLQHEIDLPPTISLSDG 120
           ESTVSGNGIPEPQVYAPPRPTDRLAVPPFAQRERFHRFQPTYPYLQHEIDLPPTISLSDG
Sbjct: 96  ESTVSGNGIPEPQVYAPPRPTDRLAVPPFAQRERFHRFQPTYPYLQHEIDLPPTISLSDG 155

Query: 121 EEPPPYQGPCTLQLRDPEQQLELNRESVRAPPNRTIFDSDLMDSARLGGPCPPSSNSGIS 180
           EEPPPYQGPCTLQLRDPEQQLELNRESVRAPPNRTIFDSDLMDSARLGGPCPPSSNSGIS
Sbjct: 156 EEPPPYQGPCTLQLRDPEQQLELNRESVRAPPNRTIFDSDLMDSARLGGPCPPSSNSGIS 215

Query: 181 ATCYGSGGRMEGPPPTYSEVIGHYPGSSFQHQQSSGPPSLLEGTRLHHTHIAPLESAAIW 240
           ATCYGSGGRMEGPPPTYSEVIGHYPGSSFQHQQSSGPPSLLEGTRLHHTHIAPLESAAIW
Sbjct: 216 ATCYGSGGRMEGPPPTYSEVIGHYPGSSFQHQQSSGPPSLLEGTRLHHTHIAPLESAAIW 275

Query: 241 SKEKDKQKGHPL 252
           SKEKDKQKGHPL
Sbjct: 276 SKEKDKQKGHPL 287
```

Schematic Colors:

Very Strong	Strong	High	Moderate	Low	Weak
>95%	80-95%	45-80%	35-45%	25-35%	20-25%

Human protein: Q8NER4 - PMEPA1 variant A protein. EMBL

Bioinformatic Harvester (c)

Idea? Comment? Inconsistency? FORUM

Length: **237 aa**, molecular weight: **26201 Da**, CRC64 checksum: **A44A274EAABFD930**

```
     MMVMVVVITC LLSHYKLSAR SFISRHSQGR RREDALSSEG CLWPSESTVS GNGIPEPQVY        60
     APPRPTDRLA VPPFAQRERF HRFQPTYPYL QHEIDLPPTI SLSDGEEPPP YQGPCTLQLR       120
     DPEQQLELNR ESVRAPPNRT IFDSDLMDSA RLGGPCPPSS NSGISATCYG SGGRMEGPPP       180
     TYSEVIGHYP GSSFQHQQSS GPPSLLEGTR LHHTHIAPLE SAAIWSKEKD KQKGHPL          237
//
```

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GoTo: SOURCE @ Stanford University

GeneReport for: AY128643

S.O.U.R.C.E. Search Help

SOURCE GeneReport: JBL suppl.

TMEPAI

transmembrane, prostate androgen induced RNA

UniGene, LocusLink, OMIM, GenAtlas, GeneCard, Ensembl, MapView, Genome Browser

Aliases

- PMEPA1
- TRANSMEMBRANE PROSTATE ANDROGEN-INDUCED RNA

Chromosomal Location

Chromosome/Cytoband	20q13.31-q13.33

Microarray Gene Expression Data

Data available	**Show Gene Expression Data**

SwissProt Information

SwissProt Accession No.	**Q969W9 Transmembrane prostate androgen-induced protein** (Homo sapiens)
Subcellular Location	type ib membrane protein (potential).
Subunit	interacts with the ww domains of nedd4 (by similarity).

Tissue Specificity	highest expression in prostate. also expressed in ovary.
Miscellaneous	alternative products: event=alternative splicing; named isoforms=2; name=1; isoid=q969w9-1; sequence=displayed; name=2; isoid=q969w9-2 sequence=vsp_006438; note=no experimental confirmation available;
Induction	by androgen.
Similarity	belongs to the tmepai family.
SwissProt Copyright	This SWISS-PROT entry is copyright. It is produced through a collaboration between the Swiss Institute of Bioinformatics and the EMBL outstation - the European Bioinformatics Institute. There are no restrictions on its use by non-profit institutions as long as its conte is in no way modified and this statement is not removed. Usage by and for commercial entities requires a license agreement (See http://www.isb-sib.ch/announce/ or send an er to license@isb-sib.ch).

Annotations

Gene Ontologies

Ontology	Annotation	Evidence	Sour
Molecular Function	Molecular function unknown	ND	GOA
Biological Process	Androgen receptor signaling pathway	NAS	GOA
Cellular Component	Integral to membrane	NAS	GOA

UniGene & EST Expression Information

UniGene Cluster — **Hs.83883 from Build No. 173 ,** Released on 2004-09-06

Normalized expression distribution for tissue type
Top ten [of 28]

[Help]

Tissue	Normalized Expression (%)	Cluster Clones Tissue clones
Pancreas:	16.86	50:67592
Soft_Tissue:	16.78	5:6792
Prostate:	9.92	42:96523
Colon:	5.82	21:82275
Embryonic Stem cells:	4.73	1:4820
Bone:	4.45	10:51252
Pooled:	4.00	1:5703
Eye:	3.18	18:128990
Muscle:	3.01	12:90826
Stomach:	2.86	9:71656

SAGE (NCBI) — Go to **Gene-to-tag Mapping** at NCBI

Upstream Genomic Sequence

TRASER — **Upstream genomic sequence** for transmembrane, prostate androgen induced RNA

Representative mRNA Sequences

UniGene — **NM_020182**

Accession	Description
NM_020182	This variant (1) represents the longest transcript and encodes the longest isoform (a).
	This variant (4) differs in the 5' UTR and coding regio compared to variant 1. The resulting isoform (c) is

LocusLink RefSeq	**NM_199171**	shorter compared to isoform a. Transcripts 3 and 4 both encode isoform c.
	NM_199169	This variant (2) differs in the 5' UTR and coding regio compared to variant 1. The resulting isoform (b) is shorter and has a distinct N-terminus compared to isoform a.
	NM_199170	This variant (3) differs in the 5' UTR and coding regio compared to variant 1. The resulting isoform (c) is shorter compared to isoform a. Transcripts 3 and 4 both encode isoform c.

Alias PubMed Search

PubMed	**Search PubMed using aliases AND** [ ] PubMed

| Search | No Clone Report | Help |

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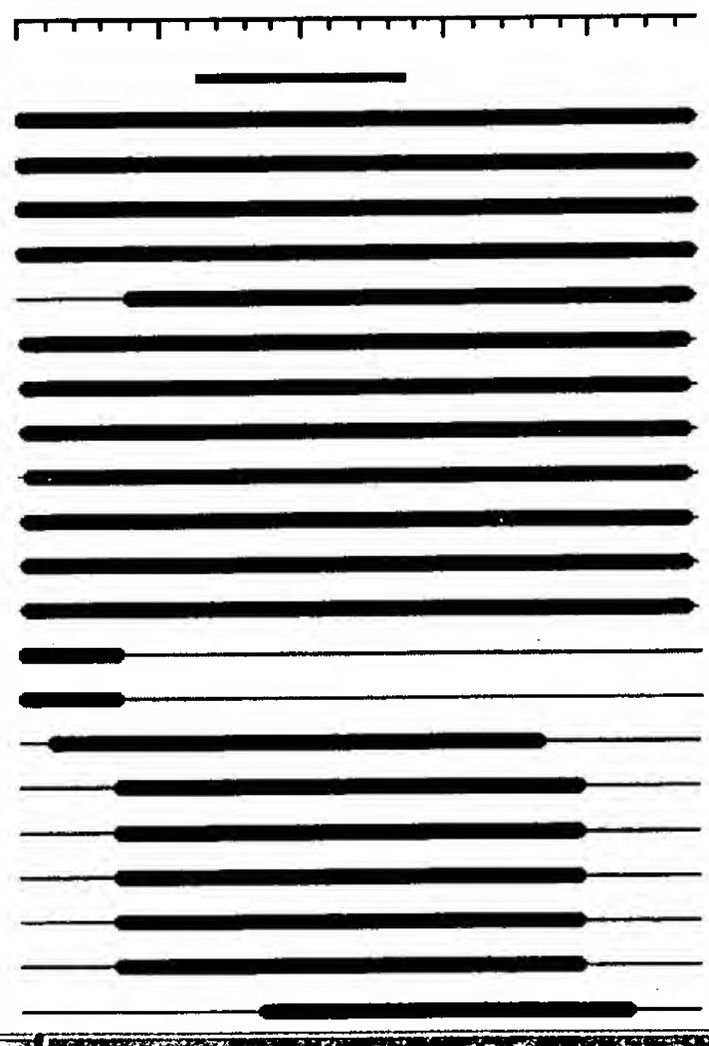
Query: gi|22121999 PMEPA1 variant A protein [Homo sapiens]
Matching gi: 40317620, 40317618

Best hits | Common Tree | Taxonomy Report | 3D structures | CDD-Search

85 BLAST hits to 24 unique species Sort by BLAST score

0 Archaea | 1 Bacteria | 61 Metazoa | 6 Fungi | 11 Plants | 0 Viruses | 6 Other Euka

Keep only [ ] Cut-Off [80] Select Reset



SCORE	P	ACCESSION	GI	PROTEIN DESCRIPTION
Conserved Domain Database hits				
1282	27	AAH15918	45946553	TMEPAI protein [Homo sa
1282	27	NP_954638	40317616	transmembrane prostate
1282	27	NP_064567	21361841	transmembrane prostate
1274	27	AAH80635	51593771	Transmembrane prostate
1096	27	CAB55862	5911816	dJ718J7.1 (androgen inc
781	27	NP_852146	41281943	chromosome 18 open reac
781	27	NP_004329	4757884	chromosome 18 open reac
781	27	NP_001003	51093708	chromosome 18 open reac
761	27	AAH66971	44890592	C18orf1 protein [Homo s
721	27	NP_852148	41281948	chromosome 18 open reac
721	27	NP_001003	51093710	chromosome 18 open reac
721	27	NP_852147	41281957	chromosome 18 open reac
186	27	CAC32857	13160408	dJ1059L7.1.2 (androgen
186	27	CAB88144	7619746	dJ1059L7.1.1 (androgen
93	27	CAD97884	31873900	hypothetical protein [H
92	27	AAQ98856	37693041	transducer of regulatec
92	27	AAH23614	33874587	MECT1 protein [Homo sap
92	27	T00388	7513044	hypothetical protein KI
92	27	AAH17075	31455200	MECT1 protein [Homo sap
92	27	NP_056136	34732709	mucoepidermoid carcinom
84	27	P54259	29429203	Atrophin-1 (Dentatorubr

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ACTIVATE: SMART analysis

No domains, repeats, motifs or features could be predicted with confidence

These features and domains are not shown in the diagram, either because their scores are less significant than the required threshold, or because they overlap with some other source of annotation:

name	begin	end	E-value	reason
Pfam:Drf_FH1	44	205	7.50e-01	threshold
IB	150	212	3.19e+03	threshold
THY	207	233	2.18e+03	threshold

SOURCE - BLAST -SMART - HomoloGene - ensEMBL - GenomeBrowser - PSORT2 - Entrez - RZPD - CDART - SOSUI - STRING top of page

UniGene Cluster Hs .83883 *Homo sapiens*

Transmembrane, prostate androgen induced RNA (TMEPAI) Links

SELECTED PROTEIN SIMILARITIES

organism, protein and percent identity and length of aligned region		
H.sapiens:	ref:NP_004329.1 - chromosome 18 open reading frame 1; clone 22 [Homo sapiens]	67.60 % / 249 aa (see ProtEST)
M.musculus:	ref:NP_075371.1 - Nedd4 WW binding# protein 4; Nedd4 WW-binding protein 4 [Mus musculus]	88.10 % / 252 aa (see ProtEST)

SOURCE - BLAST -SMART - HomoloGene - ensEMBL - GenomeBrowser - PSORT2 - Entrez - RZPD - CDART - SOSUI - STRING top of page

Ensembl **Human** *GeneView* — The Wellcome Trust Sanger Institute — EBI

Home ▸ Human ▲ What's New ▲ TextSearch ▲ BlastSearch ▲ MartSearch ▲ Export Data ▲ Download ▲ Disease

Find [All] [ ] [Lookup] [e.g. ENSG00000139618, BRCA2]

Ensembl Gene Report

Gene	**TMEPAI** (HUGO ID)
Ensembl Gene ID	**ENSG00000124225**
Genomic Location	**View gene in genomic location:** 56908875 - 56971962 bp (56.9 Mb) on chromosom **This gene is located in sequence:** AL121913.4.1.150224
Description	Transmembrane prostate androgen-induced protein (Solid tumor- associated 1 protein SWISSPROT (Q969W9)]
Prediction Method	Genes were annotated by the Ensembl automatic analysis pipeline using either a Gen from a human/vertebrate protein, a set of aligned human cDNAs followed by Genome prediction or from Genscan exons supported by protein, cDNA and EST evidence. Ge are further combined with available aligned cDNAs to annotate UTRs.
Sequence Markup	View genomic sequence for this gene with exons highlighted
Export Data	Export gene data in EMBL, GenBank or FASTA
SNP information	View information about variations on this gene.
	1: TMEPAI (ENST00000265626) [Transcript information] [Exon information] [Protein i **2:** TMEPAI (ENST00000341744) [Transcript information] [Exon information] [Protein i **3:** TMEPAI (ENST00000347015) [Transcript information] [Exon information] [Protein i

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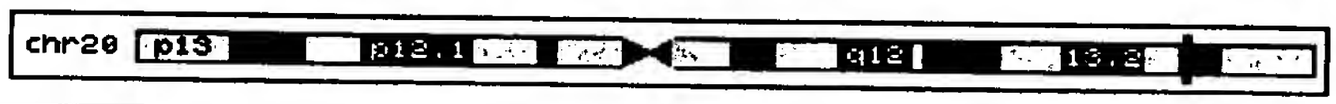
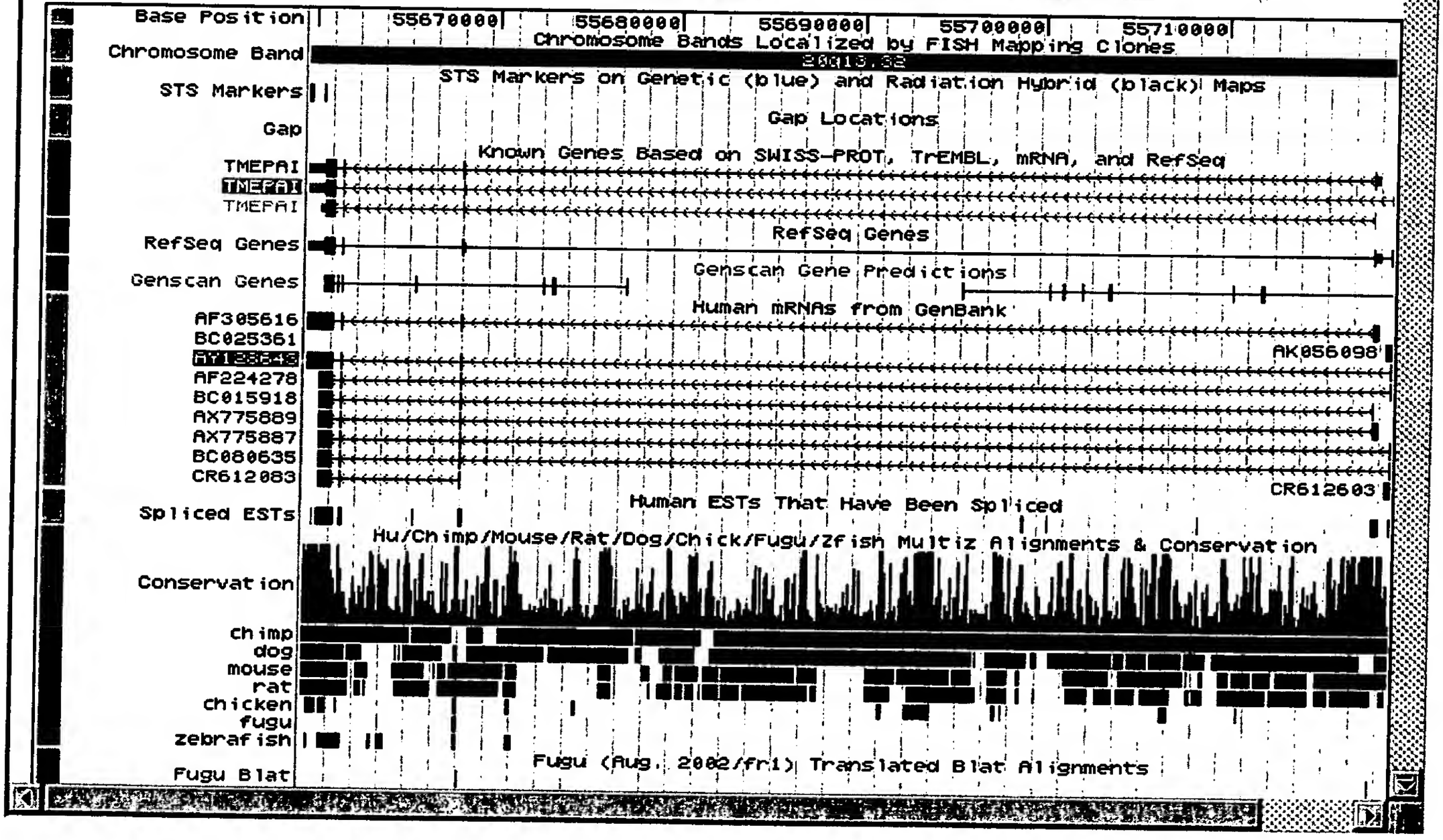
Home Genomes BLAT PCR DNA Tables Gene Sorter Convert NCBI PDF/PS Help

UCSC Genome Browser on Human May 2004 Assembly

move <<< << < > >> >>> zoom in 1.5x 3x 10x base zoom out 1.5x 3x 10x

position chr20:55,659,555-55,719,031 size 59,477 bp. image width: 620 jump





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activate PSORT II

9/23

60.9 %: nuclear
13.0 %: mitochondrial

```
          13.0 %: plasma membrane
           8.7 %: cytoplasmic
           4.3 %: vesicles of s
45
ecretory system
```

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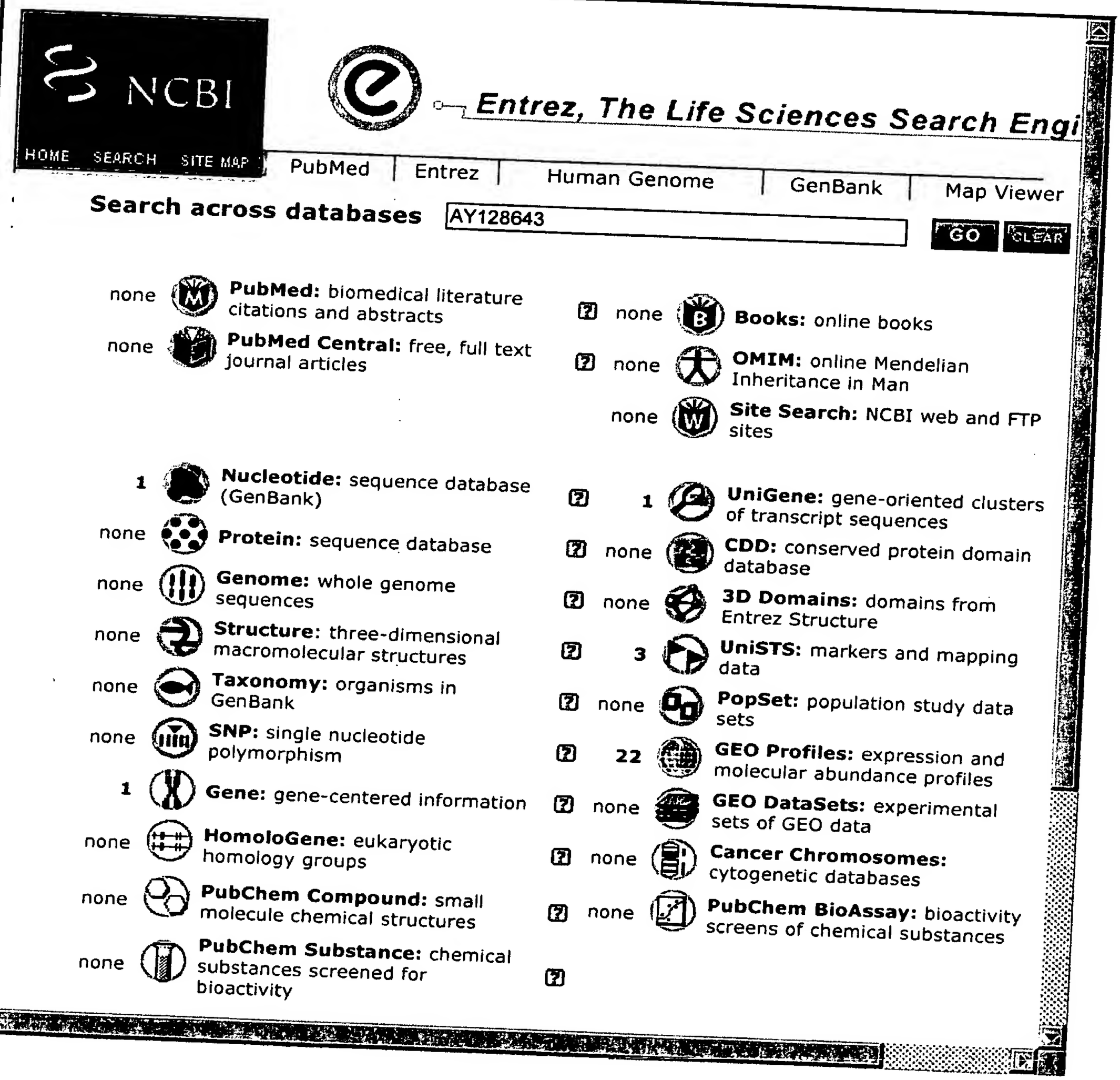

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EMBL/Genbank->Unigene mapping provided by RZPD http://www.rzpd.de

rzpd Deutsches Ressourcenzentrum für Genomforschung GmbH

Gene Information and Ordering of RZPD Products for TMEPAI (Transmembrane, prostate andro induced RNA)

NCBI Unigene Cluster Hs.83883 Homo sapiens Version : #175

Title	Transmembrane, prostate androgen induced RNA
Gene	**TMEPAI**
Locus Link	56937
Chromosome Location	20:q13.31-q13.33
RefSeq	NM_020182 NM_199169 NM_199170 NM_199171
Further Gene Links	GeneCards GeneNest S.O.U.R.C.E. SYSTERS Swiss-Pro
Cross Reference Unigene - Ensembl (Hs#24_34e)	
Ensembl Gene ID	ENSG00000124225
Complex Gene Annotation	GenomeMatrix OMIM ID:606564
Reference IDs to Unigene	Gene Symbol : TMEPAI Locus Link : 56937 RefSeq : NM_020182,NM_199169,NM_199170,NM_199171

EBI-Hinxton-"Uniprot-Swissprot-TrEMBL" database

General information

www.uniprot.org	**Q8NER4** | **XML**
Entry name	**Q8NER4**
Accession number	**Q8NER4**
Created	TrEMBLrel. 22, 1-OCT-2002
Sequence update	TrEMBLrel. 22, 1-OCT-2002
Annotation update	TrEMBLrel. 24, 1-JUN-2003

Description and origin of the Protein

Description	PMEPA1 variant A protein.
Organism source	Homo sapiens (Human).
Taxonomy	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Primates; Catarrhini; Hominidae; Homo.
NCBI TaxID	9606

References

[1] Brunschwig,E.B., Wilson,K., Mack,D., Dawson,D., Lawrence,E., Willson,J.K., Lu,S., Nosrati,A., Rerko,R.M., Swinler,S., Beard,L., Lutterbaugh,J.D., Willis,J., Platzer,P., Markowitz,S.,
PMEPA1, a transforming growth factor-beta-induced marker of terminal colonocyte differentiation whose expression is maintained in primary and metastatic colon cancer.
(2003) *Cancer Res.* **63**:1568-1575

Position SEQUENCE FROM N.A.

Medline 22557253

PubMed 12670906

Database cross-references

EMBL	AY128643; AAM89277.1; -.
GO	GO:0016021; C:integral to membrane; NAS.
	GO:0030521; P:androgen receptor signaling pathway; NAS.

SOURCE - BLAST -SMART - HomoloGene - ensEMBL - GenomeBrowser - PSORT2 - Entrez - RZPD - CDART - SOSUI - STRING

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CDART: Conserved Domain Architecture Retrieval Tool

About CDART

Sorry, there were no domain hits to your protein. Try increasing the expectation value or turn off low complexity filtering. Some proteins may not have domains defined yet.

GoTo: SOSUI analysis

This amino acid sequence is of a MEMBRANE PROTEIN
which have 1 transmembrane helix.

No.	N terminal	transmembrane region	C terminal	type	length
1	2	MVMVVVITCLLSHYKLSARSFIS	24	PRIMARY	23

SOURCE - BLAST -SMART - HomoloGene - ensEMBL - GenomeBrowser - PSORT2 - Entrez - RZPD - CDART - SOSUI - STRING

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Home · Download · Help/Info

Your Input:

ENSP00000326920 annotation not available (267 aa)

Sorry, there are no predicted associations at the current settings.

Views: Neighborhood, Fusion, Occurrence, Coexpression, Experiments, Databases

note: you bypassed the normal STRING input page.
For your information, the sequence you provided was not found as su
However, a similarity search indicated a close match to the protein 'ENSP0000032692

Info & Parameters...

Network Display - Nodes (orthologous groups) are either coloured
directly linked to the input - as in the table) or white (nodes are nc
linked to the input). Edges (putative links between orthologous grc
consist of up to three lines: a red line indicates the presence of fus
evidence for the link; green - neighbourhood evidence; blue - phyl
coocurrence evidence. A bold line indicates particularly strong evid
each case. Hover or click to reveal more information about the nod

Active Prediction Methods:

☑ Neighborhood ☑ Gene Fusion ☑ Co-occurrence
☑ Co-expression ☑ Experiments ☑ Databases ☑ Textmining

required confidence (score): medium confidence (0.400)

or custom value:

interactors shown: no more than 10 int

or custom limit:

edge scaling factor: 80%

network depth: 1

Update Parameters

[CANCER RESEARCH 63, 4299–4304, August 1, 2003]

Advances in Brief

PMEPA1, an Androgen-regulated NEDD4-binding Protein, Exhibits Cell Growth Inhibitory Function and Decreased Expression during Prostate Cancer Progression[1]

Linda L. Xu, Yinghui Shi, Gyorgy Petrovics, Chen Sun, Mazen Makarem, Wei Zhang, Isabell A. Sesterhenn, David G. McLeod, Leon Sun, Judd W. Moul, and Shiv Srivastava[2]

Center for Prostate Disease Research, Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, Maryland 20814-4799 [L. L. X., Y. S., G. P., C. S., M. M., W. Z., D. G. M., J. W. M., S. S.], and Urology Service, Walter Reed Army Medical Center [D. G. M., J. W. M.] and Department of Genitourinary Pathology, Armed Forces Institute of Pathology [I. A. S.], Washington, DC 20307

Abstract

***PMEPA1* was originally identified as a highly androgen-induced gene by serial analysis of gene expression in androgen-treated LNCaP prostate cancer (CaP) cells. *PMEPA1* expression is prostate abundant and restricted to prostatic epithelial cells. *PMEPA1*-encoded protein shows high sequence homology to a mouse *N4wbp4*-encoded protein that binds to Nedd4 protein, an E3 ubiquitin–protein ligase involved in ubiquitin-dependent, proteasome-mediated protein degradation. Studies from our and other laboratories have suggested the role of *PMEPA1* in cell growth regulation as noted by androgen induction of *PMEPA1* expression, elevated *PMEPA1* expression in nontumorigenic revertants of tumor cell lines after chromosome 8p transfer, and *PMEPA1* expression alterations (up- or down-regulation) in human tumors. Here, we demonstrate that PMEPA1 protein through its PY motifs interacts with WW domains of the human NEDD4 protein. Exogenous expression of *PMEPA1*, in widely used CaP cell lines DU145, PC3, LNCaP, and LNCaP sublines (C4, C4-2, and C4-2B), conferred cell growth inhibition, and at least one of the PY motifs of PMEPA1 may be involved in its cell growth inhibitory functions. Quantitative expression analysis of *PMEPA1* in paired normal and tumor cells of 62 patients with primary CaP revealed tumor cells associated decreased expression in 40 of 62 patients that were significantly associated with higher pathologic stage and serum prostate-specific antigen. Taken together, *PMEPA1* negatively regulates growth of androgen responsive or refractory CaP cells, and these functions may be mediated through the interaction of PMEPA1 with the NEDD4 protein involved in the ubiquitin–proteasome pathway. Loss or reduced *PMEPA1* expression in CaP further suggests for its role in prostate tumorigenesis.**

Introduction

Biological effects of androgens on target cells, *e.g.*, prostatic epithelial cell proliferation and differentiation, as well as androgen ablation-mediated cell death, involve AR-mediated[3] cell signaling (1). Systematic and comprehensive analysis of the ARGs should provide the biological reporters for androgen signaling in CaP. Our efforts to analyze ARGs by serial analysis of gene expression led to the discovery of *PMEPA1* (2, 3). *PMEPA1* expression was regulated by androgen in a dose- and time-dependent manner, and *PMEPA1* was highly expressed in the prostate in comparison with other organs.

PMEPA1 encodes a protein of 252 amino acids with a type Ib *trans*-membrane domain. PMEPA1 protein sequence homology search showed 83% identity to a recently reported mouse N4wbp4 protein, which was defined as one of the several proteins that bound to WW domain of the *Nedd4*-encoded protein (4). *Nedd4*, originally identified as a developmentally regulated gene in mice, now belongs to a family of ubiquitin–protein ligases characterized by two to four WW domains, a COOH-terminal homologous to E6-AP COOH terminus domain and an NH_2-terminal C2 domain (5–7). Additional studies implicated the roles of *Nedd4* in diverse cellular functions through the ubiquitin-dependent, proteasome-mediated protein degradation (8–12). The WW domain of the Nedd4 protein comprises of a module with two highly conserved tryptophan residues, which bind to target proteins that contain a PY motif, *e.g.*, PPxY (4, 13–17). The presence of two PY motifs in the predicted protein sequence of PMEPA1 and its similarity to the mouse Nedd4-binding protein, N4wbp4, suggest that PMEPA1 is a potential binding partner of the NEDD4, the human homologue of the Nedd4.

Studies of *PMEPA1* (2, 3, 18, 19) have suggested for its role in cell growth regulation as noted by the androgen induction of *PMEPA1* expression, elevated *PMEPA1* expression in nontumorigenic revertants of tumor cell lines after chromosome 8p transfer, and the *PMEPA1* expression alterations (up- or down-regulation) in human tumors. Furthermore, the induction of *PMEPA1* expression in nontumorigenic derivatives of multiple cancer cell lines, resulting from the introduction of chromosome 8p, suggested that *PMEPA1* might be the downstream target of the critical cell growth regulatory genes on chromosome 8, the most frequently altered chromosomes in CaP.

The androgen-regulated nature of *PMEPA1*, the potential of PMEPA1 protein as a NEDD4-binding partner and suggested cell growth regulatory functions of *PMEPA1*, have now provided the impetus to study biochemical and cell biological functions of *PMEPA1* and its CaP-associated alterations.

Materials and Methods

Plasmids. Mammalian expression vectors encoding PMEPA1-V5 and PMEPA1-GFP fusion proteins were generated by PCR amplification of the *PMEPA1* open reading frame. For *PMEPA1*-V5-pcDNA3.1 vector, the primers 5′GCTGCTGGAGAACTGAAGGCG3′ and 5′GTGTCCTTTCTGTTTATCCTTC3′ were used. For *PMEPA1*-GFP-pEGFP vector, the primers used were 5′aagcttGCTGCTGGAGAACTGAAGG CG 3′ and 5′gaattcGGTGTCCTTTCTGTTTATC3′. The V5 tag or GFP protein was fused at the COOH terminus of the PMEPA1 protein. The PCR product for generating PMEPA1-V5 was inserted into pcDNA3.1-V5-His expression vector (Invitrogen, Carlsbad, CA). The PCR product for generating PMEPA1-GFP was

Received 11/22/02; accepted 6/11/03.

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[1] Supported by a grant from the Center for Prostate Disease Research, a program of the Henry M. Jackson Foundation for the Advancement of Military Medicine (Rockville, MD), funded by the United States Army Medical Research and Materiel Command.

[2] To whom requests for reprints should be addressed, at the Center for Prostate Disease Research, Department of Surgery, Uniformed Services University of the Health Sciences, 1530 East Jefferson Street, Rockville, MD 20852. Phone: (240) 453-8952; Fax: (240) 453-8912; E-mail: ssrivastava@cpdr.org.

[3] The abbreviations used are: AR, androgen receptor; ARG, androgen-regulated gene; GFP, green fluorescence protein; CaP, prostate cancer; TSG, tumor suppressor gene; cT, cycle threshold; EGFP, enhanced green fluorescence protein; GST, glutathione *S*-transferase; TGN, *trans*-Golgi network; GAPDH, glyceraldehyde phosphate dehydrogenase; LCM, laser-capture microdissection; QRT-PCR, quantitative reverse transcription-PCR; wt, wild type; PSA, prostate-specific antigen.

digested by *Hind*III and *Eco*RI and cloned into the same sites of pEGFP vector (Clontech, Palo Alto, CA). PMEPA1-PY motif mutants, in which the tyrosine residue (Y) was replaced with an alanine residue (A), were created by using QuikChange Site-directed Mutagenesis kit (Stratagene, La Jolla, CA) and using the *PMEPA1*-V5-pcDNA3.1 vector as a template. The plasmids of *PMEPA1-PY* motif mutants are as follows: (*a*) *PMEPA1-PY1m*-V5-pcDNA3.1, with the first PY motif mutation (Y126A); (*b*) *PMEPA1-PY2m*-V5-pcDNA3.1, with the second PY motif mutation (Y197A); and (*c*) *PMEPA1-PY1m/PY2m*-V5-pcDNA3.1, with both the PY motif mutations (Y126A and Y197A). The sequences of all of the inserts in expression vectors were verified by DNA sequencing.

A bacterial expression plasmid of human *NEDD4* gene (p*NEDD4WW*-GST-pGEX-2TK) encoding all four WW-domains (accession no. XM_046129) fused to GST (GST-WW fusion protein) was generated by PCR amplification of the coding region of the four WW-domains using the primers 5′GCAG-GATCCCAACCAGATGCTGCTTGC3′ and 5′GCAGAATTCTTTTGTA-ATCCCTGGAGTA3′. Normal prostate tissue-derived cDNA was used as a PCR template, and the amplified fragment was cloned into the *Bam*HI/*Eco*RI sites of pGEX-2TK (Amersham Biotech, Piscataway, NJ). A mammalian expression vector (*NEDD4*-GFP-pEGFP) encoding NEDD4-GFP fusion protein was generated using the primers 5′GCAAAGCTTGTCCGG TTTGCTG-GAAGC3′ and 5′GCAGAATTCCCTTTTTGTTCTTATTGGTGAC3′ to generate the *NEDD4* gene fragment by PCR.

PMEPA1 and NEDD4 Protein-binding Assays. The *in vitro* binding of PMEPA1 and NEDD4 was assessed by GST pull-down assays. GST-WW fusion protein was prepared and purified with glutathione-Sepharose beads per Amersham Biotech instructions. [^{35}S]methionine-labeled proteins representing PMEPA1 and its mutants were generated by *in vitro* transcription/translation (TNT T7 quick coupled transcription/translation system; Promega, Madison, WI). Briefly, the *PMEPA1*-V5-pcDNA3.1 or three mutants (2 μg) were incubated in 40 μl of reticulocyte lysate with 40 μCi of [^{35}S]methionine for 1.5 h at 30°C. [^{35}S]methionine incorporation into protein was measured, and samples were equalized on the basis of cpm. The GST-WW fusion protein bound to glutathione-Sepharose beads (5 μg) was incubated with the [^{35}S]methionine-labeled lysates (12 μl) in 0.4 ml of PBS (pH 7.4), 1 mM DTT, and protease inhibitors. The negative control for each [^{35}S]methionine-labeled lysate represented a reaction mixture with equivalent amounts of the lysate incubated with glutathione-Sepharose beads without GST-WW fusion protein. After 16 h of incubation at 4°C, the beads were washed six times with PBS, resuspended in SDS-PAGE sample buffer, and run on 12% SDS-PAGE gel under a reducing condition. The gels were dried and autoradiographed.

The interaction of PMEPA1 and NEDD4 proteins in cells was evaluated by a coimmunoprecipitation assay. 293 cells (human embryonal kidney cells) were cotransfected with *NEDD4*-GFP-pEGFP vector and one of the *PMEPA1*-V5 expression vectors encoding either wt PMEPA1-V5 or the PY mutants of *PMEPA1*. Thirty-six h later, the cells were collected and lysed, and the lysates were immunoprecipitated with anti-GFP antibody (Clontech) following the manufacturer's protocol. The immunoprecipitated proteins were subjected to immunoblotting with an anti-V5 tag antibody (Invitrogen).

Immunofluorescence Assays. These experiments were performed following the procedure described by Harvey *et al.* (17). Briefly, stable transfectants of LNCaP cells harboring *PMEPA1*-GFP-pEGFP (LNCaP-*PMEPA1*-GFP transfectant) were grown on coverslips for 2 days, fixed in 2% paraformaldehyde for 15 min, and permeabilized in 0.2% Triton X-100 for 2 min. Fixed and permeabilized cells were incubated with anti-GM130 (recognizes a *cis*-Golgi matrix protein) or anti-TGN38 (recognizes a protein localizing to TGN) monoclonal antibodies (BD Transduction Laboratory, San Diego, CA) at 6.25 μg/ml for 30 min at room temperature. Cells were then washed to remove excess or nonspecifically bound primary antibody followed by incubation with tetramethylrhodamine isothiocyanate-conjugated antimouse antibody (Sigma, St. Louis, MO) at 1:100 dilution for 30 min at room temperature. The sections were mounted with fluoromount (Southern Associates, Birmingham, AL), and the images were processed with a Leica fluoromicroscope and Open-Lab software (Improvision, Lexington, MA).

Colony Formation Assays and Cell Proliferation Analysis. Prostate cell lines LNCaP, PC3, and DU145 were purchased from American Type Culture Collection (Rockville, MD) and grown in the cell culture media as described by the supplier. The LNCaP sublines C4, C4-2, and C4-2B (20–22) were purchased from Urocor (Oklahoma, OK) and cultured in T medium (5% fetal bovine serum, 80% DMEM, 20% F12, 5 μg/ml insulin, 13.65 pg/ml Triiodothyronine, 5 μg/ml apo-transferrin, 0.244 μg/ml biotin, and 25 μg/ml adenine). Three μg of plasmids (*PMEPA1*-V5-pcDNA3.1 or vector without *PMEPA1* insert) were transfected into the 50–70% confluent cells in triplicate in 60-mm Petri dishes with Lipofectamine (Invitrogen). TSG *p53* (wt) and mt p53 (R175H and G245D) were also used in parallel as controls. Approximately 36 h later, selection with G418 at 800 μg/ml (DU145 and PC3) or 400 μg/ml (LNCaP and its sublines) was initiated. Cells were maintained with G418-containing medium that was changed every 3–4 days. After 2–4 weeks of selection, the cells were rinsed with 1× PBS, fixed with 2% formaldehyde in 1× PBS for 15 min, stained with 0.5% crystal violet in 1× PBS for 15 min,

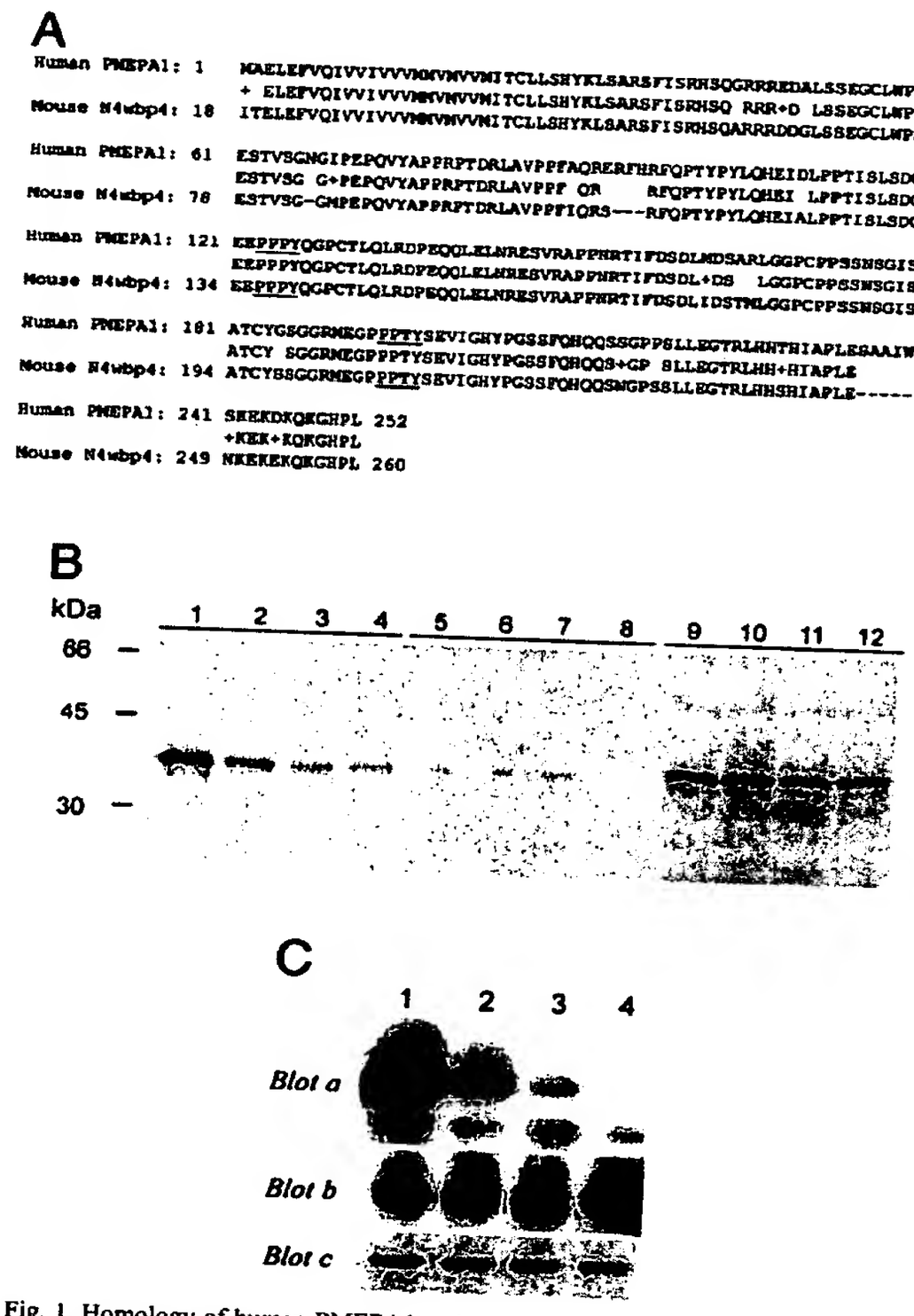

Fig. 1. Homology of human PMEPA1 and mouse N4wbp4 protein sequences and the interaction of PY motifs of PMEPA1 with the WW domains of NEDD4. In *A*, PMEPA1 and N4wbp4 (GenBank accession no. AK008976) exhibit 83% sequence identity (analyzed through the National Center for Biotechnology Information Web site).[4] The + denotes conservative substitution. The PY motifs are *underlined*. *B*, *in vitro* transcription/translation products ([^{35}S]Methionine-labeled lysates) derived from expression plasmids. *PMEPA1*-V5-pcDNA3.1 (*Lanes 1* and *5*), *PMEPA1-PY1m*-pcDNA3.1 (*Lanes 2* and *6*), *PMEPA1-PY2m*-pcDNA3.1 (*Lanes 3* and *7*), and *PMEPA1-PY1m/PY2m*-pcDNA3.1 (*Lanes 4* and *8*) were incubated with GST-NEDD4-WW-Sepharose beads (*Lanes 1–4*) or control GST beads (*Lanes 5–8*), and [^{35}S]Methionine-labeled proteins bound to GST-NEDD4-WW-Sepharose beads were solubilized in sample buffer and resolved by SDS-PAGE gel. Equal amounts of [^{35}S]Methionine lysates corresponding to samples in *Lanes 1–4* were run on SDS-PAGE gel without GST pull-down (*Lanes 9–12*). In *C*, 293 cells were cotransfected with expression vectors encoding NEDD4-GFP and one of the following fusion proteins: PMEPA1-V5 (*Lane 1*), PMEPA1-PY1m-V5 (*Lane 2*), PMEPA1-PY2m-V5 (*Lane 3*), or PMEPA1-PY1m/PY2m-V5 (*Lane 4*). The cell lysates from each group were immunoprecipitated with anti-GFP antibody and then subjected to immunoblotting (*Blot a*). Cell lysates from each group without immunoprecipitation were also processed for immunoblotting (*Blots b* and *c*) to serve as control. *Blots a* and *b* were detected by anti-V5 antibody, and *Blot c* was detected by anti-GFP antibody.

[4] Internet address: www.ncbi.nlm.nih.gov.

and rinsed one to two times with distilled H_2O. Colonies visible in each dish without magnification were counted by Open-Lab software. To assess the effects of PY motif mutations on the colony-forming ability of *PMEPA1*, LNCaP and PC3 cells were also transfected with *PMEPA1* mutants: *PMEPA1-PY1m*-pcDNA3.1, *PMEPA1-PY2m*-pcDNA3.1, or *PMEPA1-PY1m/PY2m*-pcDNA3.1. *PMEPA1*-V5-pcDNA3.1 and expression vector without insert served as positive and negative controls, respectively, for the *PMEPA1* mutants. Two independent colony-forming assays were performed as above.

To further evaluate the growth inhibitory effects of *PMEPA1* on CaP cells, a stable *PMEPA1*-GFP-Tet LNCaP transfectant was generated. Expression of PMEPA1-GFP fusion protein in these cells was negatively regulated by tetracycline in the medium (Clontech). For cell proliferation assays, 3000 *PMEPA1*-GFP-Tet LNCaP cells were seeded in 96-well plates with or without 1 μg/ml tetracycline in the medium. CellTiter 96 Aqueous One Solution kit (Promega) was used to measure the cell proliferation according to the manufacturer's instructions.

Prostate Tissue Specimens, LCM, and QRT-PCR Assay. Matched CaP and normal tissues were derived from radical prostatectomy specimens from 62 CaP patients treated at Walter Reed Army Medical Center (under an Institutional Review Board-approved protocol). The procedures for collecting specimens were described previously (23). Ten-μm frozen sections were prepared and archived at -70°C. Histologically normal prostate epithelial and prostate tumor cells from each patient were harvested by a pathologist (W. Z.) using LCM equipment according to the protocol provided by the manufacturer (Arcturus Engineering, Mountain View, CA). Total RNA was prepared from the harvested normal and tumor prostate epithelial cells as described previously (23) and quantified with Fluorometer (Bio-Rad, Hercules, CA). QRT-PCR was conducted using 0.1 ng of total RNA from paired normal and tumor cells. *PMEPA1* PCR primers were carefully designed that only amplify *PMEPA1* but not *STAG1*, an alternatively spliced form of *PMEPA1* (19). The PCR primers were 5′CATGATCCCCGAGCTGCT3′ and 5′TGATCTGAACAAACTCCAGCTCC3′, and the FAM-labeled probe was 5′AGGCGGACAGTCTCCTGCGAAAC3′. GAPDH gene expression was detected as the internal control (PE Applied Biosystems, Foster, CA). Paired triplicate samples (one lacking reverse transcriptase and duplicate with reverse transcriptase) were amplified in 50-μl volumes containing the manufacturer's recommended universal reagent, proper primers, and probe of *PMEPA1* or *GAPDH* using 7700 sequence detection system (PE Applied Biosystems). Results were plotted as average cT values for each duplicate sample minus the average duplicate cT values for *GAPDH*. Differences between matched tumor (T) and normal (N) samples were calculated using $2\exp(cT_{tumor} - cT_{normal})$ and expressed as fold changes in expression. The expression status of *PMEPA1* was further categorized as overexpression in tumor tissue (T > N), defined as 1+ (1.5–3-fold), 2+ (3.1–10-fold), 3+ (10.1–20-fold), and 4+ (>20-fold) increased expression as compared with matched normal tissue; reduced expression in tumor tissue (T < N), defined as 1− (1.5–3-fold), 2− (3.1–10-fold), 3− (10.1–20-fold), and 4− (>20-fold) decreased expression as compared with matched normal tissue; and no change (T = N) refers to the difference of *PMEPA1* expression between T and N as <1.5 folds (0). No detectable *PMEPA1* expression in one of the specimens of tumor/normal pairs was scored as 4+ for increased or 4− for decreased expression.

Statistical Analysis. Statistical analysis was performed with the SPSS software package. The association between *PMEPA1* expression and clinicopathological features was analyzed using χ^2 and t tests. $P < 0.05$ was considered as statistically significant.

Results and Discussions

PMEPA1-PY Motifs Interact with the WW Domains of NEDD4. The WW domains of NEDD4 protein facilitate its binding to the target proteins via interaction with the PY motifs of NEDD4-binding proteins (4, 13–17). Predicted PMEPA1 protein sequence comprises of two PY motifs, PY1 (PPPY) and PY2 (PPTY). PY1 is in the central region of the PMEPA1 protein, and PY2 is close to the COOH terminus of the PMEPA1 protein (Fig. 1*A*). PMEPA1 shares 83% sequence identity with the protein encoded by *N4wbp4*, a gene expressed in mouse embryo (Ref. 4; Fig. 1*A*). *In vitro* translated [^{35}S]Methionine-labeled PMEPA1-V5 fusion protein, with the two intact PY motifs, showed binding to the GST-WW fusion protein (Fig. 1*B*, *Lane 1*). PMEPA1 with PY1 or PY2 mutations revealed significantly decreased binding to WW domains (Fig. 1*B*, *Lanes 2* and *3*). Furthermore, PMEPA1-V5 and NEDD4-GFP fusion proteins expressed in 293 cells showed strong association (Fig. 1*C*, *Lane 1*) and the mutant PMEPA1-V5 proteins having single mutation of PY1 or PY2 motif or double mutations of both PY1 and PY2 motifs exhibited significantly reduced binding to NEDD4 (Fig. 1*C*, *Lanes 2–4*). Thus, both *in vitro* and cell culture data support the prediction that PMEPA1 interacts with NEDD4, and these interactions are dependent on the binding of both PY motifs of PMEPA1 to WW domains. However, the PY2 motif mutation appeared to have a greater effect on binding of PMEPA1 to NEDD4 WW domain.

The high protein sequence identity of PMEPA1 with N4wbp4 suggests that *PMEPA1* is the human homologue of *N4wbp4*. On the basis of the suggested role of Nedd4 in mouse development (4–12), the homology of PMEPA1 to a Nedd4-binding protein, experimental documentation of binding of PMEPA1 to NEDD4, and the androgen regulation and prostate abundance of PMEPA1 warrant evaluations of the roles of *PMEPA1* in prostate development.

PMEPA1 Is a Golgi-associated Protein. To gain additional insights into PMEPA1 cellular functions, its subcellular localization was determined. PMEPA1-GFP fusion protein showed perinuclear localization with a Golgi-like appearance (Fig. 2*A*). We performed the immunofluorescence assay to test the hypothesis that PMEPA1 local-

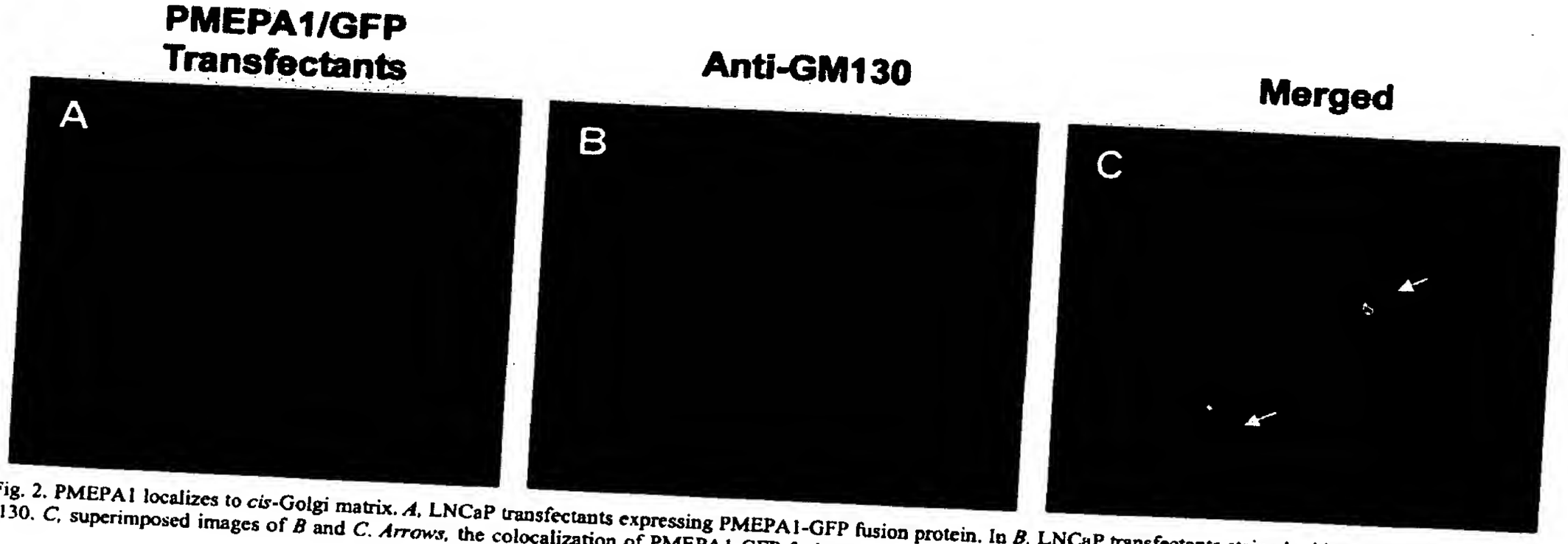

Fig. 2. PMEPA1 localizes to *cis*-Golgi matrix. *A*, LNCaP transfectants expressing PMEPA1-GFP fusion protein. In *B*, LNCaP transfectants stained with anti-Golgi structure protein GM130. *C*, superimposed images of *B* and *C*. *Arrows*, the colocalization of PMEPA1-GFP fusion protein and GM130. Magnification: ×100.

izes to the Golgi complex. The images of subcellular location of GM130, a *cis*-Golgi protein, showed a similar pattern as PMEPA1-GFP fusion protein (Fig. 2*B*). Superimposition of the images of PMEPA1-GFP fusion protein and GM130 in LNCaP-PMEPA1-GFP transfectants confirmed the localization of PMEPA1-GFP fusion protein on *cis*-Golgi structure (Fig. 2*C*). We did not see the colocalization of PMEPA1-GFP and TGN-38, which localizes to TGN (data not shown). In this regard, the subcellular localization of *PMEPA1* is similar to other two newly identified Nedd4 WW domain-binding proteins, N4wbp5 and N4wbp5a, which were also localized to the Golgi complex (12, 17). N4wbp5a was observed to sequester the trafficking of Nedd4/Nedd4–2, thereby increasing the activity of the epithelial sodium channel, a known target down-regulated by *NEDD4* (12). As a highly ARG and NEDD4-binding protein, the localization of PMEPA1 on Golgi apparatus may suggest for the role of *PMEPA1* in the regulation of protein turnover of AR targets.

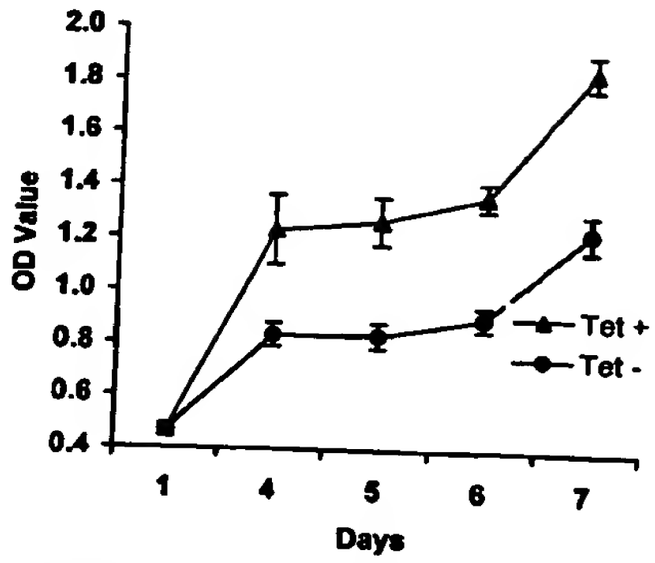

Fig. 4. Effect of *PMEPA1* on cell proliferation. Stable *PMEPA1*-GFP-Tet-LNCaP transfectants (described in "Materials and Methods") were seeded in 96-well plates with or without 1 μg/ml tetracycline in the medium. The cell proliferation was measured using the CellTiter 96 Aqueous One Solution kit at the indicated time. *Tet* + and *Tet* −, the cell culture medium with or without tetracycline, respectively. The absorbance values which reflected the cell numbers are significantly different ($P < 0.01$) between the two groups, except on day 1.

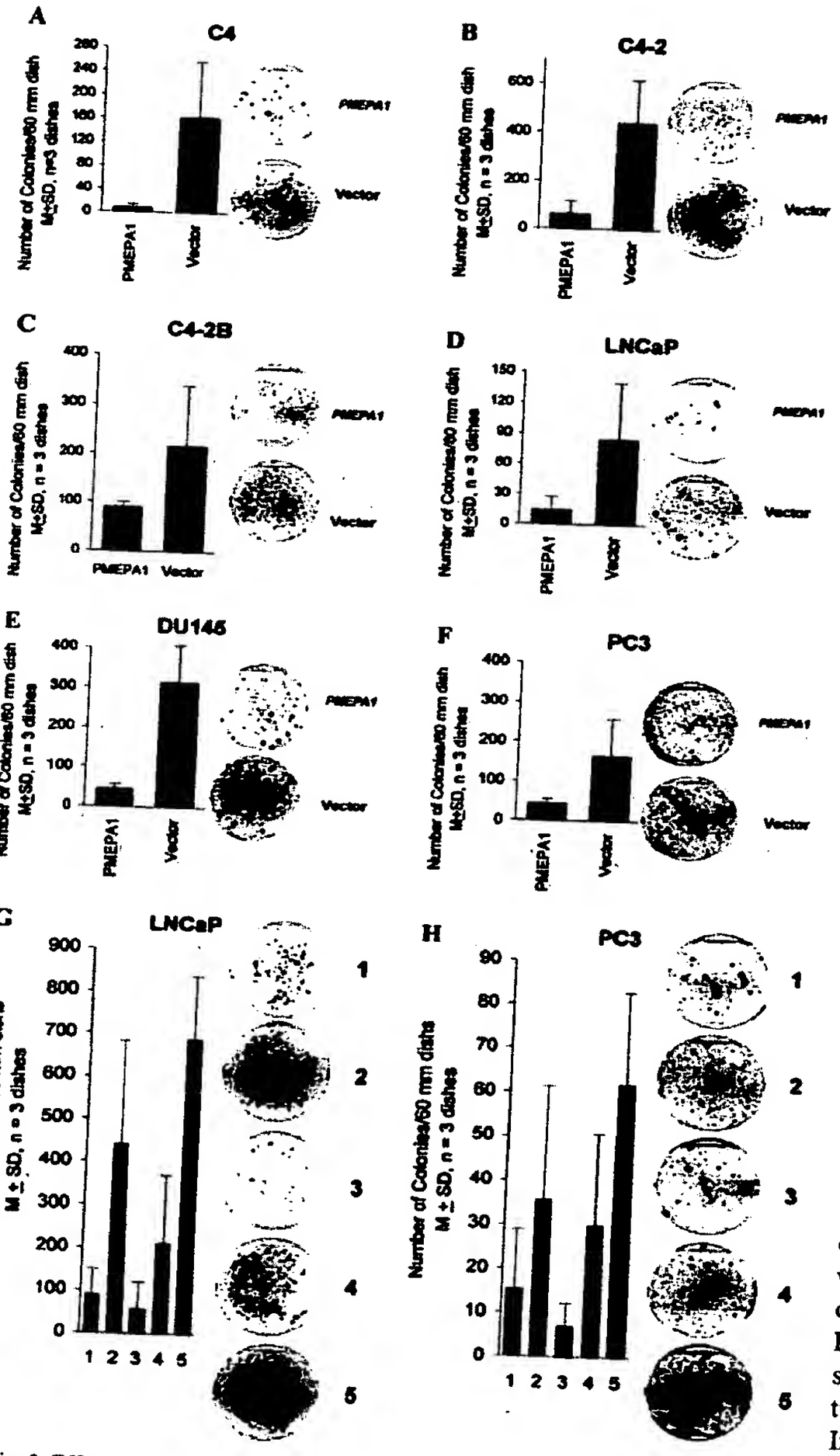

Fig. 3. Effect of *PMEPA1* on colony formation. Prostate tumor cell lines C4 (*A*), C4-2 (*B*), C4-2B (*C*), LNCaP (panel *D*), DU145 (*E*), and PC3 (*F*) were transfected with 3 μg each of *PMEPA1*-V5-pcDNA3.1 (*PMEPA1*) and pcDNA3.1 vector (Vector) in triplicate sets. In separate experiments, LNCaP (*G*) and PC3 (*H*) cells were transfected with control vector or expression vectors encoding wt-PMEPA1 or PMEPA1-PY mutants (*1*, *PMEPA1*-V5-pcDNA3.1; *2*, *PMEPA1-PY1m*-pcDNA3.1; *3*, *PMEPA1-PY2m*-pcDNA3.1; *4*, *PMEPA1-PY1m/PY2m*-pcDNA3.1; *5*, pcDNA3.1). Transfected cells were selected for plasmid-containing cells with G418 for 3 weeks, and surviving cells were fixed and stained with crystal violet. Colonies were counted and displayed as histograms. For each cell line, a photograph of one dish of cells treated with 3 μg of each plasmid is also shown.

Table 1 *PMEPA1 expression status in primary prostate cancers*

PMEPA1 gene expression in 62 matched LCM-derived prostatic cancer cells (T) and normal cells (N) was analyzed by QRT-PCR. The results were represented as differential expression in T and N as described in "Materials and Methods." T < N, reduced expression in tumor tissue, defined as 1− (1.5–3-fold), 2− (3.1–10-fold), 3− (10.1–20-fold), and 4− (>20-fold) decreased expression as compared with matched normal tissue; T > N, overexpression in tumor tissue, defined as 1+ (1.5–3-fold), 2+ (3.1–10-fold), 3+ (10.1–20-fold), and 4+ (>20-fold) increased expression as compared with matched normal tissue; and T = N, similar expression between T and N, refers to the difference of *PMEPA1* expression between T and N as <1.5-fold (0).

PMEPA1 expression	No. of patients/group (%)	Degree of *PMEPA1* expression	
		Quantity	No. (%)
T < N	40 (64.5)	1−	11 (27.5)
		2−	17 (42.5)
		3−	5 (12.5)
		4−	7 (17.5)
T > N	10 (16.1)	1+	6 (60.0)
		2+	4 (40.0)
		3+	
		4+	
T = N	12 (19.4)	0	

PMEPA1 Inhibits Growth of CaP Cells. To investigate the biological effects of *PMEPA1* expression in the regulation of cell growth and contribution of PY motifs for such functions, we performed the colony formation assay by transfection expression vectors of the wt-*PMEPA1* and *PMEPA1-PY* mutants. As shown in Fig. 3, *A–F*, colony-forming abilities of CaP cell lines DU145, PC3, LNCaP, and LNCaP sublines were significantly suppressed by transfection of the sense version of wt-*PMEPA1* expression vector. Under these conditions, wt-p53 showed similar cell growth inhibition (data not shown). In two independent experiments, mutations of PY1 motif appear to abolish the inhibition of colony formation by wt-*PMEPA1*, emphasizing the role of PY1 motif in PMEPA1 and NEDD4 interactions and the biological functions of *PMEPA1* (Fig. 3, *G* and *H*). It is intriguing that both PY1 and PY2 mutations diminished the *in vitro* binding of PMEPA1 to NEDD4; however, in the context of biological function of *PMEPA1* in the colony-forming assay, only mutation of PY1 motif has functional consequence. The growth inhibitory effect of *PMEPA1* appears to be linked to the interactions of PY1 motif to NEDD4 WW

Table 2 *PMEPA1 expression and clinicopathological features*

The patients were grouped into two groups: T < N ($n = 40$), *PMEPA1* expression reduced ≥1.5-fold in tumor cells in comparison with matched normal cells, and T ≥ N, *PMEPA1* expression was similar between tumor and normal cells ($n = 12$) or increased in tumor cells ($n = 10$).

PMEPA1 expression	Pathologic stage (%)		PSA range (%)			PSA recurrence (%)		Time to recurrence after surgery (month)
	T2	T3	≤4 ng/ml	4.1–10 ng/ml	10.1–20 ng/ml	No	Yes	Mean ± SE
T < N	11 (27.5)	29 (72.5)	1 (2.5)	30 (75.0)	9 (22.5)	29 (72.5)	11 (27.5)	8.2 ± 3.4
T ≥ N	12 (54.5)	10 (45.5)	5 (22.7)	15 (68.2)	2 (9.1)	19 (86.4)	3 (13.6)	18.4 ± 6.3
P	0.035			0.023		0.211		0.18

domain. This interpretation is based on the striking observations showing distinctively more colonies with PY1 motif mutant in comparison with wt-*PMEPA1*. Moreover, the growth inhibitory effect of *PMEPA1* has been confirmed by the cell proliferation characteristics of stable *PMEPA1*-GFP-Tet-LNCaP cells, where exogenous *PMEPA1* is up-regulated in the absence of tetracycline. The growth of the *PMEPA1*-GFP-Tet LNCaP cells in tetracycline-negative medium is significantly slower than that of PMEPA1-tet LNCaP transfectant in tetracycline-positive medium (Fig. 4). LNCaP cells with *PMEPA1* overexpression also revealed increased retinoblastoma phosphorylation, further confirming cell growth inhibitory effect of *PMEPA1* (data not shown).

PMEPA1 is expressed in AR-positive CaP cell lines: LNCaP and its sublines (C4, C4-2, and C4-2B). LNCaP cells are androgen dependent for growth. Although the growth of LNCaP sublines is androgen independent, AR is critical for their proliferation (24). We observed that overexpression of *PMEPA1* by transfecting the *PMEPA1* expression vector into LNCaP and its sublines significantly inhibited the cell proliferation. Because our preliminary observations showed that *PMEPA1* overexpression in LNCaP cells resulted in altered expression of AR downstream genes,[5] we hypothesized that the growth inhibitory effect of *PMEPA1* on LNCaP and its sublines may be mediated through directly or indirectly affecting AR functions. Despite the growth inhibitory effect on AR-positive CaP cell lines, *PMEPA1* was also found to inhibit the growth of AR-negative prostate tumor cells, DU145 and PC3, suggesting that the growth inhibitory effects of *PMEPA1* on DU145 and PC3 could be mediated through alternative mechanisms, *e.g.*, regulation of other nuclear steroid receptors by *PMEPA1*. Nonetheless, inhibition of CaP cell growth by *PMEPA1* suggested that *PMEPA1* might be involved in CaP development.

Decreased *PMEPA1* Expression in Prostate Tumor Tissues. CaP cell growth inhibitory functions of *PMEPA1* led us to carefully evaluate the relationship of *PMEPA1* expression alterations to the clinicopathologic features of CaP. The overall expression pattern of the *PMEPA1* is shown in Table 1. Comparison of *PMEPA1* expression between tumor and normal cells revealed tumor cell-associated decreased expression (T < N) in 64.5% tumor specimens (40 of 62), increased expression (T > N) in 16.1% specimens (10 of 62), and no change (T = N) in 19.4% specimens (12 of 62). When these expression patterns were stratified by organ-confined (pT2) and nonorgan-confined (pT3) disease, a higher percentage of *PMEPA1* reduction was seen in pT3 (74%) *versus* pT2 (48%). Because the T > N group has a few cases, we combined the T > N and T = N groups (T ≥ N group). Comparison of the clinicopathologic parameters between the *PMEPA1*-T < N and *PMEPA1*-T ≥ N groups revealed that the *PMEPA1*-T < N group had a significantly higher percentage of patients with pT3 tumors ($P = 0.035$), and more patients in this group had a higher level of preoperative serum PSA ($P = 0.023$; Table 2). Of 62 patients whose tumors were analyzed for *PMEPA1* expression, 14 patients showed CaP recurrence as defined by serum PSA level ≥ 0.2 ng/ml after prostatectomy. Of the 14 patients, 11 showed reduced tumor-associated *PMEPA1* expression (78.5%). Reduced *PMEPA1* expression seems to associate with a higher recurrence rate and shorter duration to recurrence after surgery, although the statistical analysis did not reveal a significant difference which might be attributable to the small number of patients (Table 2). It is worth noting these observations in the context of the report showing consistent high levels of induction of *PMEPA1* in nontumorigenic revertants of tumor cell lines after the transfer of human chromosome 8p (18), which harbors putative TSGs (25, 26). Among chromosomal "hot spots" in CaP and other cancers, 8p loss is particularly frequent, occurring in ~80% of prostate tumors (27–29). Transfer of single human chromosome 8 has been shown to result in suppression of the malignant phenotype or the metastatic ability in a variety of cell lines (18, 30, 31). Therefore, it is possible that the reduced expression of *PMEPA1* in prostate tumors might result from the loss of the putative TSGs or cell growth inhibitory genes on chromosome 8p in prostate tumor cells. Two very recent interesting reports have described *PMEPA1* as a transforming growth factor-β-induced gene and marker of terminal colonocyte differentiation (32, 33).

In summary, we have demonstrated that PMEPA1 interacts with NEDD4 through its PY motifs. *PMEPA1* has cell growth inhibitory effects when overexpressed in androgen-dependent or -independent CaP cells. PY1 motif of PMEPA1 appears to modulate its cell growth inhibitory function. *PMEPA1* may function in the regulation of protein turnover via ubiquitination/proteasome pathways in cells. The biological effects of *PMEPA1* in CaP cells and expression pattern of *PMEPA1* in CaP indicate that *PMEPA1* may function as the cell growth inhibitor regulated by androgen.

Acknowledgments

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MOLECULAR CARCINOGENESIS 32:44–53 (2001)

Characterization of a Novel Gene, *STAG1/PMEPA1*, Upregulated in Renal Cell Carcinoma and Other Solid Tumors

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Using differential display–polymerase chain reaction, we identified a novel gene sequence, designated solid tumor–associated gene 1 (*STAG1*), that is upregulated in renal cell carcinoma (RCC). The full-length cDNA (4839 bp) encompassed the recently reported androgen-regulated prostatic cDNA *PMEPA1*, and so we refer to this gene as *STAG1/PMEPA1*. Two *STAG1/PMEPA1* mRNA transcripts of approximately 2.7 and 5 kb, with identical coding regions but variant 3′ untranslated regions, were predominantly expressed in normal prostate tissue and at lower levels in the ovary. The expression of this gene was upregulated in 87% of RCC samples and also was upregulated in stomach and rectal adenocarcinomas. In contrast, *STAG1/PMEPA1* expression was barely detectable in leukemia and lymphoma samples. Analysis of expressed sequence tag databases showed that *STAG1/PMEPA1* also was expressed in pancreatic, endometrial, and prostatic adenocarcinomas. The *STAG1/PMEPA1* cDNA encodes a 287-amino-acid protein containing a putative transmembrane domain and motifs that suggest that it may bind src homology 3– and tryptophan tryptophan domain–containing proteins. This protein shows 67% identity to the protein encoded by the chromosome 18 open reading frame 1 gene. Translation of *STAG1/PMEPA1* mRNA in vitro showed two products of 36 and 39 kDa, respectively, suggesting that translation may initiate at more than one site. Comparison to genomic clones showed that *STAG1/PMEPA1* was located on chromosome 20q13 between microsatellite markers *D20S183* and *D20S173* and spanned four exons and three introns. The upregulation of this gene in several solid tumors indicated that it may play an important role in tumorigenesis. © 2001 Wiley-Liss, Inc.

Key words: differential display; polymerase chain reaction; 20q13; gene expression

INTRODUCTION

Tumorigenesis is the result of many genetic alterations, which act coordinately to contribute to the disease process. Identification of the molecular events of tumor progression is crucial to providing targets for cancer prevention and treatment. Such techniques as differential display (DD)–polymerase chain reaction (PCR) [1] are valuable tools for isolating disease-associated genes. For example, DD-PCR has been used extensively to identify genes that are expressed differentially in a range of cancers, including breast, prostate, and ovarian cancer [2–4]. Although a number of genes associated with renal cell carcinoma (RCC) have been identified using DD-PCR [5–8], their precise role in RCC tumorigenesis is yet to be elucidated.

To identify additional genes that are expressed differentially in RCC, DD-PCR was performed to compare RNA derived from RCC with RNA from normal kidney parenchyma obtained from the same person. Using this approach, we pinpointed 14 genes differentially expressed in RCC [9]. One of these novel partial gene sequences was upregulated in RCC and was chosen for further analysis. In this study, we further characterized this gene. The full-length cDNA encompassed the recently reported androgen-regulated prostatic *PMEPA1* cDNA [10]. We showed that this gene not only was overexpressed in RCC but also was upregulated in other solid tumors. Because our analyses indicated that there was virtually no expression in leukemia and lymphoma samples, we termed this gene solid tumor – associated gene 1 (*STAG1*), and we now suggest that a more appropriate name may be *STAG1/PMEPA1*.

MATERIALS AND METHODS

Clinical Samples

Kidney tissue was collected at the time of nephrectomy from patients with RCC at the Princess

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Abbreviations: DD, differential display; PCR, polymerase chain reaction; RCC, renal cell carcinoma; *STAG1*, solid tumor–associated gene 1; nr, nonredundant; EST, expressed sequence tag; RT, reverse transcription; UTR, untranslated region; C18orf1, chromosome 18 open reading frame 1; SH3, src homology 3; WW, tryptophan tryptophan.

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Alexandra Hospital, Brisbane, Australia, and stored at −80°C. Histologic assessment of tumor tissue from the 16 patients participating in this study confirmed clear cell RCC, based on the Heidelberg classification of renal cell tumors [11], with a tumor stage of T1 or T2, N0, M0 (stage I or II), as determined by the tumor, node, metastasis (TNM) staging of RCC [12]. Informed consent was obtained in all cases. Ethics approval was obtained from both the Queensland University of Technology and Princess Alexandra Hospital ethics committees.

DD-PCR, Identification, and Characterization of the *STAG1/PMEPA1* cDNA and Gene

DD-PCR was performed using a Delta Differential Display kit (Clontech, Palo Alto, CA) according to the manufacturer's protocol, with modifications as described previously [9,13]. Duplicate paired RCC and normal kidney samples from four different patients were analyzed using 65 different primer combinations. A 434-bp cDNA, identified as being upregulated in all four patient's RCC samples, was cloned into pGEM-T Easy (Promega, Madison, WI) and sequenced. This sequence then was used to screen the National Centre for Biotechnology Information GenBank nonredundant (nr) and expressed sequence tag (EST) databases. Matching clones (accession nos. AI446289, AI521341, AI587542, AI677810, and AI972096) were purchased (Incyte Genomics, Palo Alto, CA) and sequenced. Additional sequencing, to generate a complete contig, was obtained by PCR using the following primers: 5′-GGATAAACAGAAAGGACACCC-3′ (forward) and 5′-AGGCGACTCTGAAATTCCG-3′ (reverse), after reverse transcription (RT) of oligo(dT)-primed RCC total RNA.

In Vitro Transcription and Translation

Two templates, for use in in vitro transcription/translation experiments, were generated by PCR from EST clone AI677810 using forward primers 5′-GGATCCTAATACGACTCACTATAGGGAGACCAC-CATCGTCCATGCACCGCTTGATGG -3′ and 5′-GGATCCTAATACGACTCACTATAGGGAGACCAC-CATGTTCCAGAGCATGGAGATCACG-3′ (T7 promoter sequence underlined) and reverse primer 5′-GGACCCTAGAGAGGGTGTCC-3′. The fidelity of these PCR products was confirmed by sequencing. Each PCR product (1 μg) was transcribed and translated in vitro using a TNT T7 Coupled Reticulocyte Lysate System (Promega) in the presence of ^{35}S-methionine (Amersham, Piscataway, NJ). Control reactions were performed using luciferase cDNA and no DNA. Protein products were separated by electrophoresis on a 12.5% Tris polyacrylamide gel. The gel was fixed, washed in Amplify Reagent (Amersham), dried overnight, and exposed to X-ray film (AGFA Curix, Brisbane, Australia) for 2 h.

Northern Analysis

RCC and paired normal kidney total RNAs (20 μg) were separated by denaturing gel electrophoresis and transferred to a Hybond N+ nylon membrane (Amersham). Human Multiple Tissue Northern blots and Matched Tumor/Normal Expression arrays were purchased from Clontech. *STAG1/PMEPA1* cDNA (nucleotides 4429–4763), cloned into pGEM-T Easy (Promega), was linearized with SalI before transcription with T7 RNA polymerase, generating an antisense [α-^{32}P]UTP-labeled cRNA probe using a StripEz RNA kit (Ambion, Austin, TX). Hybridizations were performed at 68°C overnight in Ultrahyb solution (Ambion) and washed to a final stringency of 0.1× sodium chloride, sodium citrate/0.1% sodium dodecyl sulfate at 72°C. The Human Multiple Tissue Northern blots and the Matched Tumor/Normal array also were probed with a cRNA probe generated from EST clone AI972096 (nucleotides 1–1403 of the *STAG1/PMEPA1* cDNA), which had been linearized with SalI. Blots were reprobed with β-actin cDNA to confirm RNA loadings.

Semiquantitative RT-PCR

Semiquantitative RT-PCR was performed on RCC and matching normal kidney tissue from 12 patients (six males and six females), using the following PCR primers: 5′-CCAGAACCATAAAAT-ATCCCG-3′ and 5′-CAACAAGCAGTTTCTTCAGG-CC-3′. Optimal cycling parameters, shown to be in the linear range of amplification, were 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min for 26 cycles, followed by a 7-min extension at 72°C. A control PCR also was performed for β_2-microglobulin for 25 cycles.

RESULTS

Identification and Characterization of *STAG1/PMEPA1* cDNA and Protein

Using a modified DD-PCR technique, a novel 434-bp cDNA (Figure 1A), subsequently designated *STAG1*, was identified as being upregulated in RCC (data not shown). This partial cDNA, which showed no homology to any known genes, contained a polyadenylation signal consensus sequence (AATAAA) [14]. An additional sequence was obtained from clones identified by searching the EST database. The complete 5′ (nucleotides 1–1659) and 3′ (nucleotides 1848–4839) ends of the cDNA were obtained from clones AI677810 and AI446289, respectively. Sequence spanning these clones (nucleotides 1156–1899) was amplified from RCC total RNA by RT-PCR. From these sequences, a 4839-bp contig (Figure 1A) of the *STAG1* cDNA sequence was generated and submitted to GenBank under the accession no. AF305616.

The complete cDNA contained 320 bp of 5′ untranslated region (UTR) and a 3′ UTR of 3658 bp,

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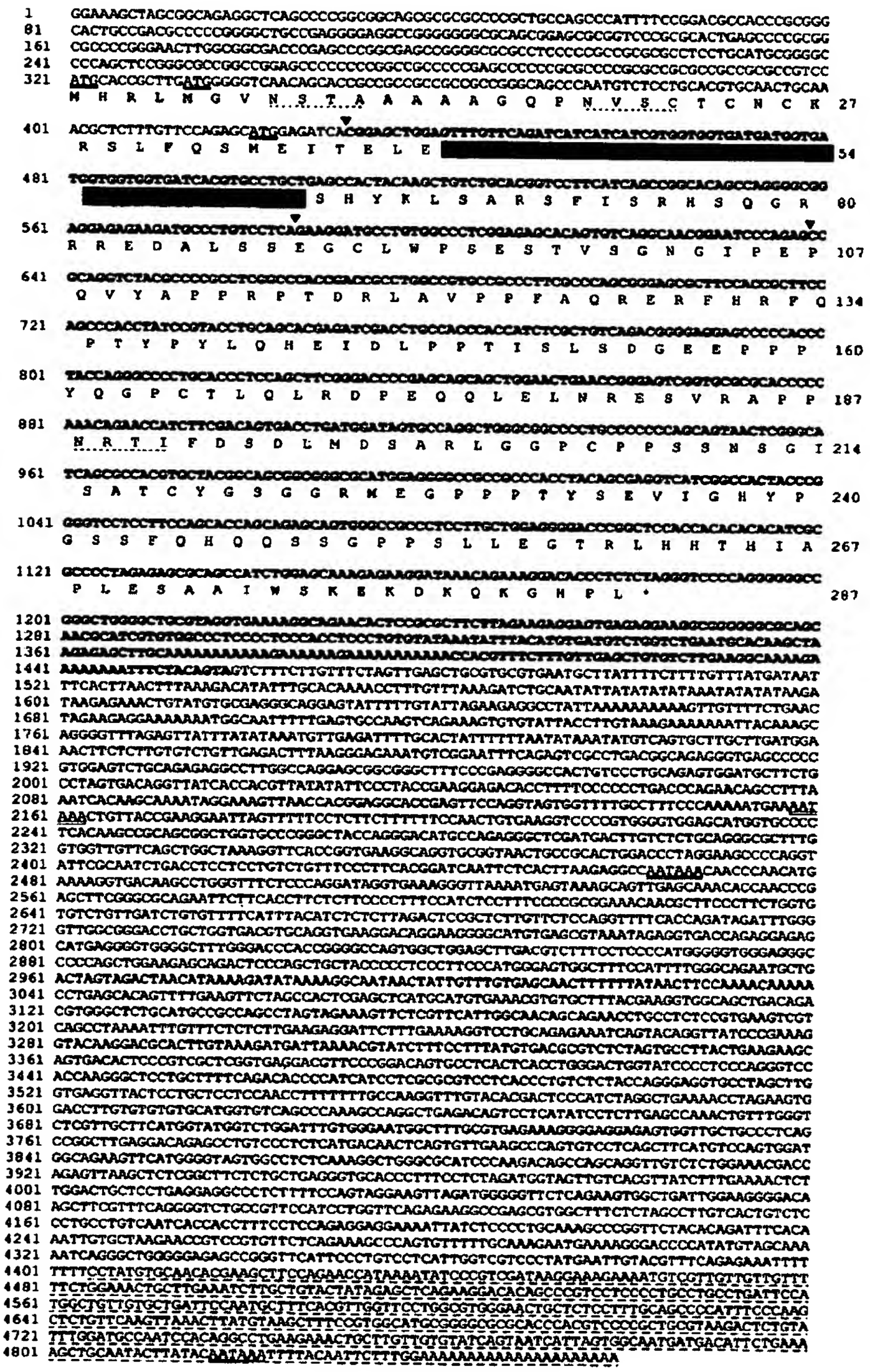

Figure 1. (*Continued next page*)

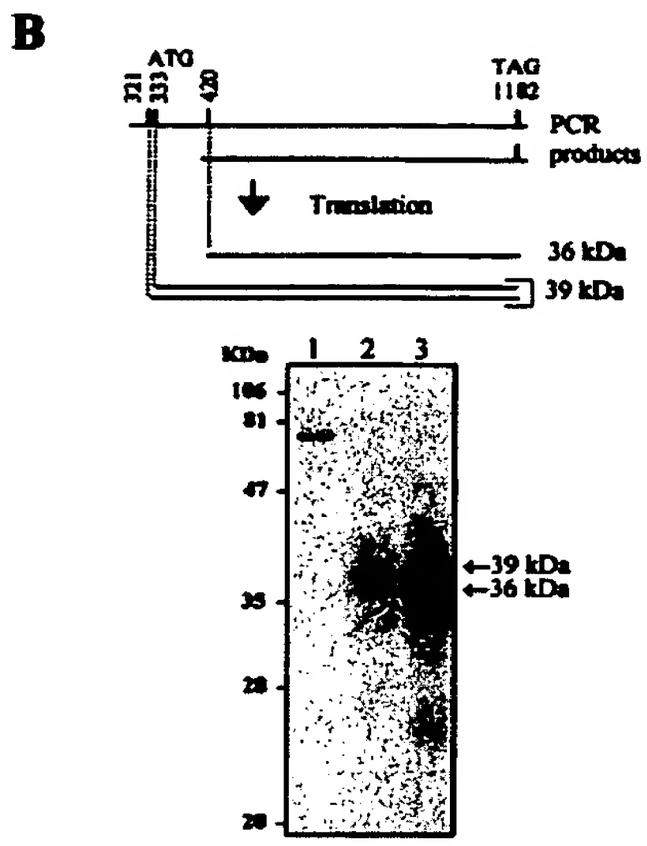

Figure 1. (A) Nucleotide and amino acid sequence of human STAG1/PMEPA1. Nucleotides are numbered on the left and amino acids on the right. Exon/intron boundaries are marked with arrowheads. Three closely spaced start codons at positions 321–323, 333–335, and 420–422 are underlined with solid lines, and consensus polyadenylation signals at positions 2158, 2463, and 4818 are underlined with two solid lines. The PMEPA1 nucleotide sequence [10] is marked in bold, and the STAG1 fragment identified by DD-PCR is marked by broken underlining. The potential transmembrane domain is boxed in gray. Consensus *N*-glycosylation sites at N^{8}, N^{19}, and N^{188} are marked by wavy underlining in the protein sequence. (B) In vitro transcription/translation of *STAG1/PMEPA1* cDNA. Schematic representation of the PCR products used for in vitro transcription/translation and the protein products generated. Lane 1, control reaction from luciferase cDNA, giving a protein product of the predicted size (61 kDa); lane 2, proteins generated from the PCR product, containing nucleotides 313–1189 of the *STAG1/PMEPA1* cDNA; lane 3, protein generated from the PCR product, containing nucleotides 413–1189 of the *STAG1/PMEPA1* cDNA. A negative control reaction lacking DNA also was performed and was clear of reaction products (data not shown). Molecular weights are indicated. (C) Hydrophobicity plot of the deduced STAG1/PMEPA1 protein. The method of Kyte and Doolittle [16] was used with a window of 17 residues. The peak corresponds to amino acids 41–62 and represents the putative transmembrane domain. [Figure can be viewed in color in the online version of this article, available at www.interscience.wiley.com]

with the largest open reading frame of 861 bp (nucleotides 321–1181), and encoded a 287-amino-acid protein of 32 kDa. Consensus polyadenylation signals (AATAAA) were located at nucleotides 2158, 2463, and 4818 (Figure 1A). The *STAG1* cDNA encompassed 1042 nucleotides (nucleotides 430–1458) of the recently reported *PMEPA1* cDNA [10] (Figure 1A), and therefore this gene will be referred to as *STAG1/PMEPA1*. This gene also showed homology (77%), at the nucleotide level, over the coding region of chromosome 18 open reading frame 1 (*C18orf1*) cDNA [15].

The *STAG1/PMEPA1* cDNA contained three closely spaced methionine codons (Figure 1A), which may be capable of functioning as sites for translation initiation. In vitro transcription/translation from two PCR products, containing nucleotides 313–1189 and 413–1189 of the *STAG1/PMEPA1* cDNA, was performed to determine which of the first/second or third ATG codons would be functional in an in vitro system. In vitro transcription/translation from the larger PCR product generated two protein products of approximately 36 and 39 kDa, whereas the shorter PCR product generated a single protein of approximately 36 kDa (Figure 1B). These data indicated that the ATG codons at 321–323/333–335 and 420–422 are capable of functioning as initiating methionines in vitro. It was not possible to determine from these experiments whether translation from the first or second or both of these ATG codons generated the 39-kDa protein.

To gain insight into the potential role of *STAG1/PMEPA1*, the deduced protein sequence was analyzed for cellular sorting signals, potential functional and structural domains, and sequence homologies. This analysis indicated that STAG1/PMEPA1 lacked consensus signals for both secretion and translocation to the nucleus. However, identification of a hydrophobic region spanning amino acids 41–62 [16], which had the characteristics of a type Ib transmembrane domain (Figure 1C), indicated that STAG1/PMEPA1 was a putative transmembrane protein with the amino terminus located extracellularly and the carboxy terminus located intracellularly. Although a search of the PROSITE database [17] showed that the deduced STAG1/PMEPA1 protein does not contain any known functional or structural domains, several proline-rich motifs were identified that may function as

binding sites for src homology 3 (SH3) and tryptophan tryptophan (WW) domains [18]. The motifs PPRP (amino acid 112–115), PTYP (amino acid 135–138), and PCPP (amino acid 205–208) conform to the consensus for binding of the SH3 domains (PXXP). In addition, the motifs PPPY (amino acid 158–161) and PPTY (amino acid 229–232) correspond to WW domain consensus binding sites. Futhermore, five putative casein kinase II (amino acids 37, 151, 190, 231, and 254) and four potential protein kinase C (amino acids 68, 116, 182, and 199) phosphorylation sites were detected, along with three consensus motifs for *N*-linked glycosylation (N-X-T/S) at N^{8}, N^{19}, and N^{188} (Figure 1A) and two potential *N*-myristoylation sites (amino acids 6 and 213). Searches of the GenBank nr database indicated that STAG1/PMEPA1 protein showed significant homology (67%) only to the C18orf1 protein.

Genomic Structure of *STAG1/PMEPA1*

The *STAG1/PMEPA1* gene sequence was contained within the genomic clones RP5-1059L7, RP5-1007E6, and RP4-718J7 (accession nos. AL121913, AL161943, and Al035541, respectively), which were identified by screening the GenBank nr database (Figure 2A). These clones are located on chromosome 20q13 between microsatellite markers D20S183 and D20S173. The *STAG1/PMEPA1* gene was approximately 62 kb in length and contained five exons and three introns. Exons ranged in size from 54 to 4201 bp and introns from 434 to 49 775 bp. Intron/exon junctions were determined by comparison of cDNA and genomic sequences (Figure 2B). All of the intron/exon junctions conform to the GT-AG rule. Interestingly, the *STAG1/PMEAP1* gene contained two alternatively spliced first exons — exon 1A and exon 1B (Figure 2A). The exon 1A described by Xu et al. [10] also was contained within the genomic clone RP5-1059L7 but was 562 bp further upstream from the exon 1 sequence descibed here (exon 1B). The use of the alternative exon 1A sequence gives rise to a smaller protein, two amino acids shorter than the smallest protein described in Figure 1A (from the third ATG). The *STAG1/PMEPA1* gene sequence, encompassing exon 1B to exon 4, was submitted to Genbank under the accession no. AF305426.

STAG1/PMEPA1 Expression Pattern in Normal and Malignant Tissues

Northern blot analysis of 16 normal human tissues using a cRNA probe containing the coding region of *STAG1/PMEPA1* (nucleotides 1–1403) showed two transcripts of approximately 5 and 2.7 kb that were highly expressed in the prostate, as previously reported [10]. Lower levels were observed in the ovary, and very low expression was noted in heart, lung, skeletal muscle, pancreas, small intestine, and colon (Figure 3A). When probed with a cRNA probe derived from the 3′ UTR of *STAG1/PMEPA1*, only the 5 kb transcript, which showed the same expression pattern, was noted (Figure 3A). Because the *STAG1/PMEPA1* cDNA (4839 bp), together with a polyadenylation tail, was approximately the same size as the 5-kb transcript seen on Northern analysis, it is likely that we have identified the complete *STAG1/PMEPA1* coding sequence. The smaller, 2.7-kb transcript most likely results from use of the polyadenylation signal at nucleotide 2465 (Figure 1A). To extend the expression analysis, a multiple tissue expression array containing polyA + RNA from 76 normal human tissues and cell lines was probed with the 5′ end of the *STAG1/PMEPA1* cRNA probe. Confirming the results of Northern blot analysis, significant levels of *STAG1/PMEPA1* mRNA were detected in the prostate (no. 53) (Figure 3B). Significant levels of expression also were detected in putamen (no. 16), aorta (no. 23), and the lung carcinoma cell line A549 (no. 69) (Figure 3B). While there was moderate expression of *STAG1/PMEPA1* in the majority of tissues arrayed on the blot, there was virtually no expression in the leukemia (nos. 62, 64, and 65) and lymphoma (nos. 66 and 67) samples (Figure 3B).

To confirm the upregulation of this gene in RCC, as shown by DD-PCR, Northern analysis and semiquantitative RT-PCR were performed. Northern analysis of three paired RCC and normal samples showed that *STAG1/PMEPA1* mRNA was detected only in RCC (data not shown). To examine a larger number of samples, semiquantitative RT-PCR was performed on another 12 RCC and normal kidney paired samples. Upregulation of *STAG1/PMEPA1* in RCC was confirmed in 10 of the 12 (83%) paired samples (Figure 3C). Together with the DD-PCR results, *STAG1/PMEPA1* was upregulated in 14 of 16 (87%) paired RCC samples.

Analysis of the source of the ESTs matching the *STAG1/PMEPA1* sequence showed that this gene also was expressed in a wide variety of other malignancies, such as pancreatic, endometrial, stomach, and prostate adenocarcinoma. To determine whether *STAG1/PMEPA1* is upregulated in any other malignancies, we analyzed a matched tumor/normal expression array containing cDNA from 68 tumor and corresponding normal tissues from individual patients with a variety of different cancers (kidney, breast, prostate, uterus, ovary, cervix, colon, lung, stomach, rectum, and small intestine). Confirming our semiquantitative RT-PCR and Northern blot analysis results, 10 of 14 kidney cancers (all clear cell RCCs ranging from stage II to IV) showed more intense hybridization than their normal kidney counterparts (Figure 4A). Seven of eight stomach adenocarcinomas and five of seven rectal adenocarcinomas showed significantly higher levels of expression of *STAG1/PMEPA1* compared with the corresponding normal samples (Figure 4B

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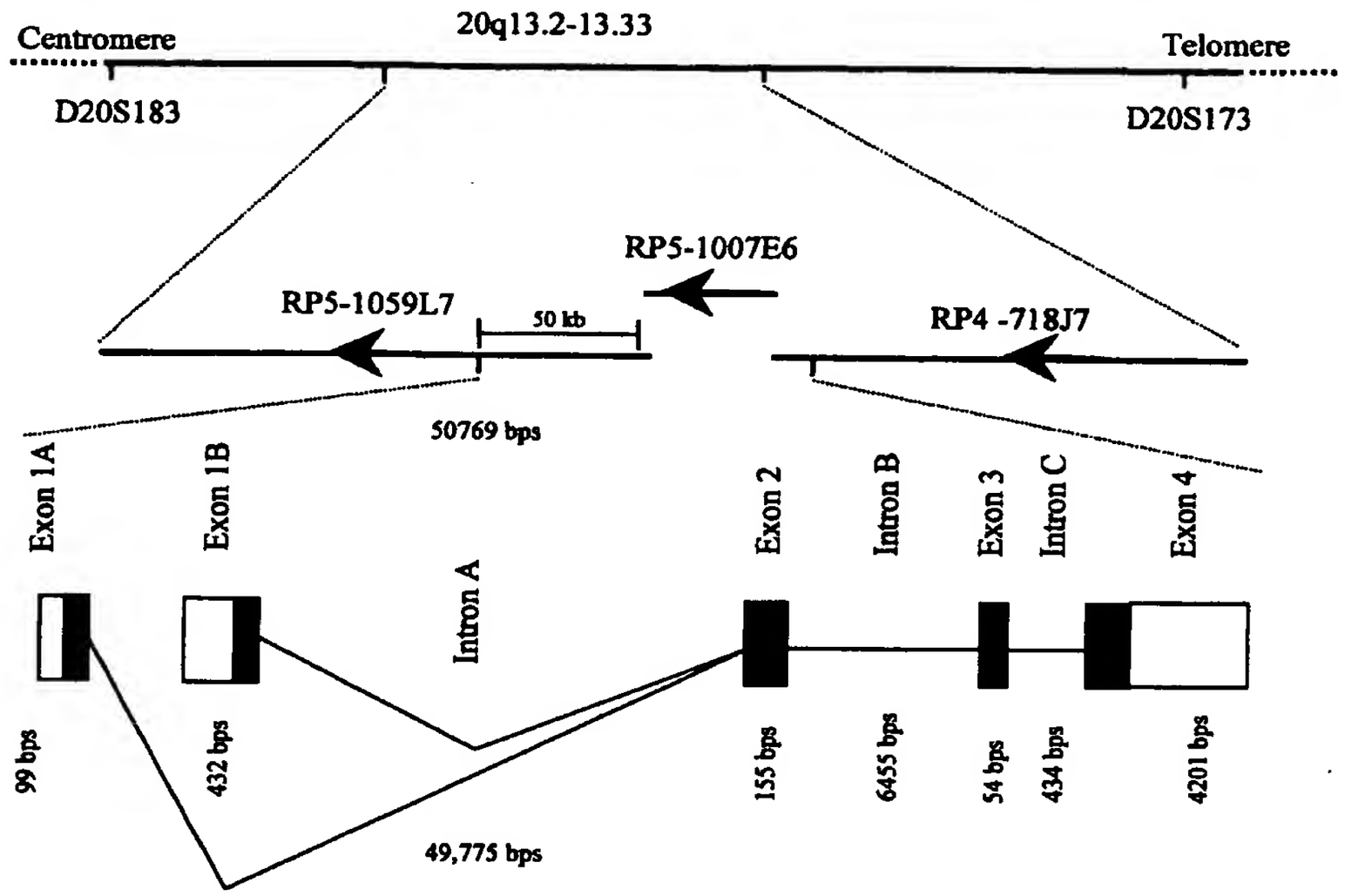

B

Sequence at exon-intron junction					
Exon/ Intron	3'-splice acceptor	Exon size (bp)	5'-splice donor	Intron size(bp)	Amino acids interrupted
1A/A	-	>432	ATGGAGATCA^gtgagtgacc	49,775	Ile-T/hr-Glu
1B/A	-	99	CAGGCAATGG^gtgatccatc	50,769	Met-A/la-Glu
2/B	gtctccacag^CGGAGCTGGA	155	CCTGTCCTCA^gtaagtgccc	6455	Ser-/-Glu
3/C	tcctgtgcag^GAAGGATGCC	54	AATCCCAGAG^gtgagacctc	434	Glu-/-Pro
4	cttcctccag^CCGCAGGTCT	4201	-	-	-

Figure 2. (A) *STAG1/PMEPA1* gene location and structure. The *STAG1/PMEPA1* gene was located on human chromosome 20q13 between microsatellite markers *D20S183* and *D20S173* and spanned the genomic clones RP5-1059L7, RP5-1007E6, and RP4-718J7 (accession nos. AL121913, AL161943, and AI035541, respectively). Arrows represent the direction of the genomic clones. Exons are represented as filled boxes and untranslated regions as unfilled boxes; introns are given as lines (not to scale). Exon and intron sizes are indicated. (B) Exon/intron boundaries of the *STAG1/PMEPA1* gene. Exon and intron sequences are shown in uppercase and lowercase letters, respectively. The nucleotide consensus sequence of the intron adjoining the splice junctions are shown in boldface type.

and C). There was no consistent differential expression patterns in the other paired normal and malignant samples from other tissues.

DISCUSSION

To date, a small number of studies have examined RCC using the DD-PCR technique [5–8]. A number of interesting differentially expressed genes have been identified from these studies, but their precise role in RCC tumorigenesis is yet to be elucidated. Using a modified DD-PCR technique, we showed that a diverse range of genes is upregulated in RCC [9]. Characterization of a novel sequence upregulated in RCC showed that this gene also had increased expression in solid tumors of the stomach and rectum but was not expressed in leukemia and

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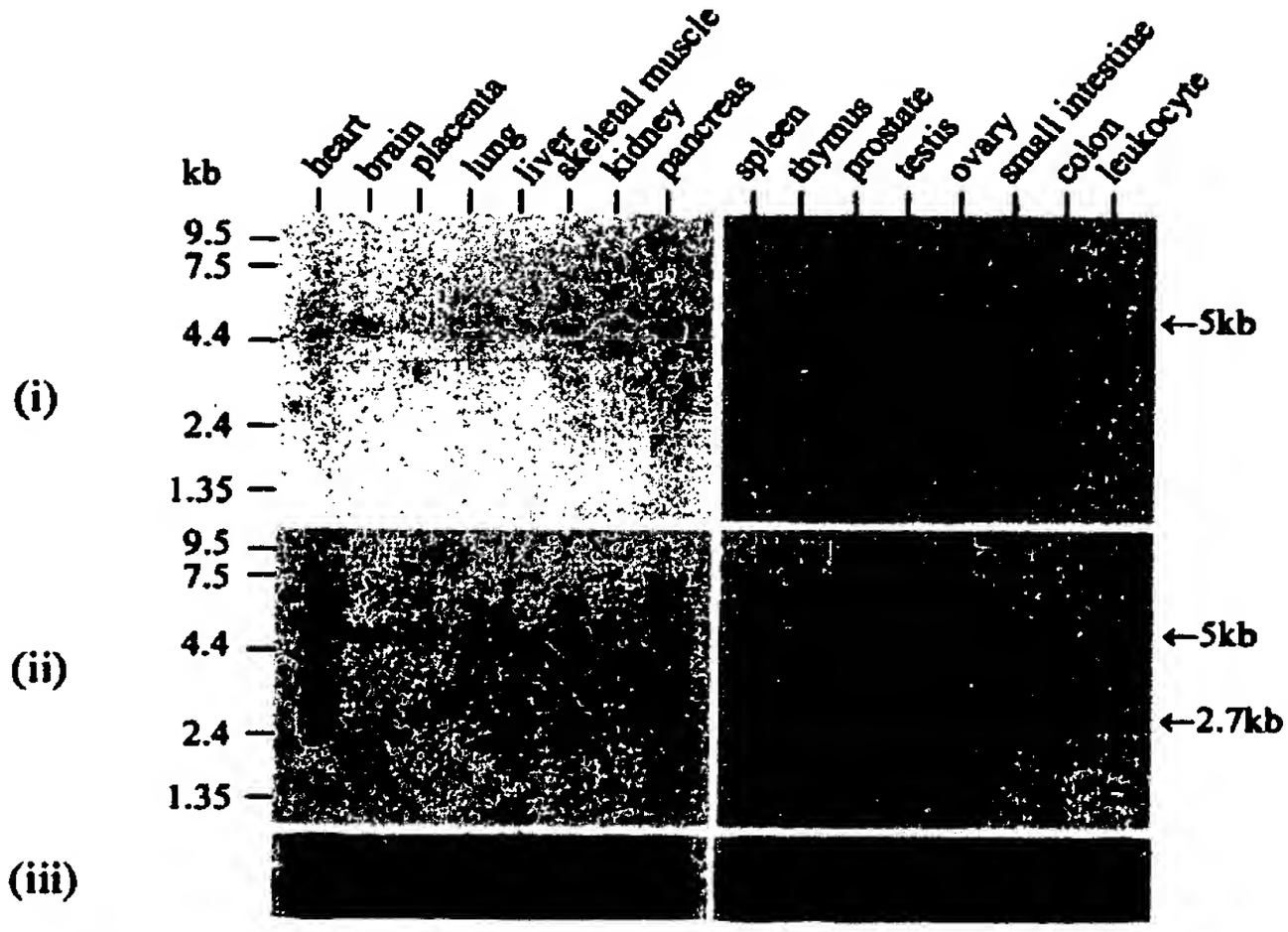
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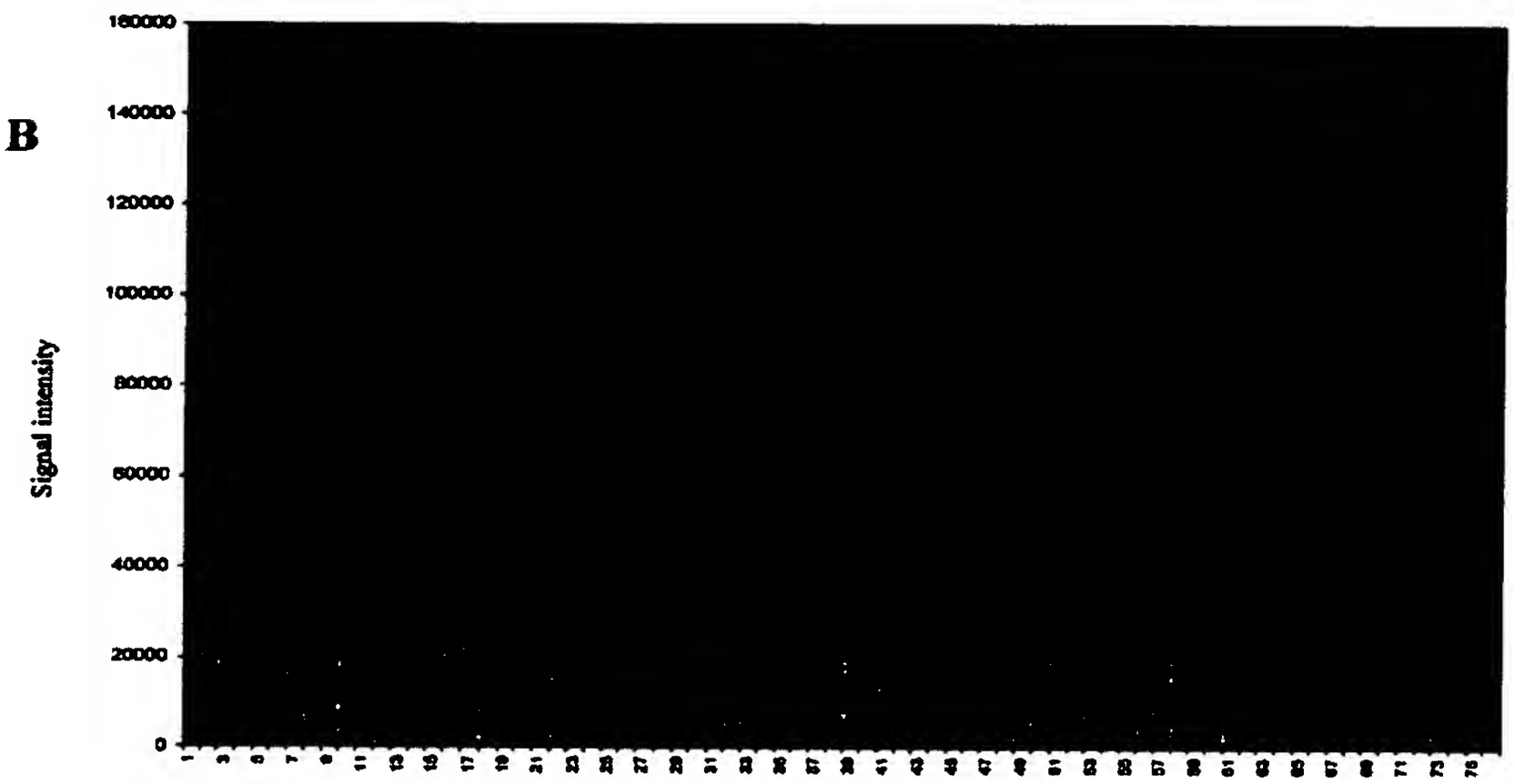
B


160000
140000
120000
100000
80000
60000
40000
20000
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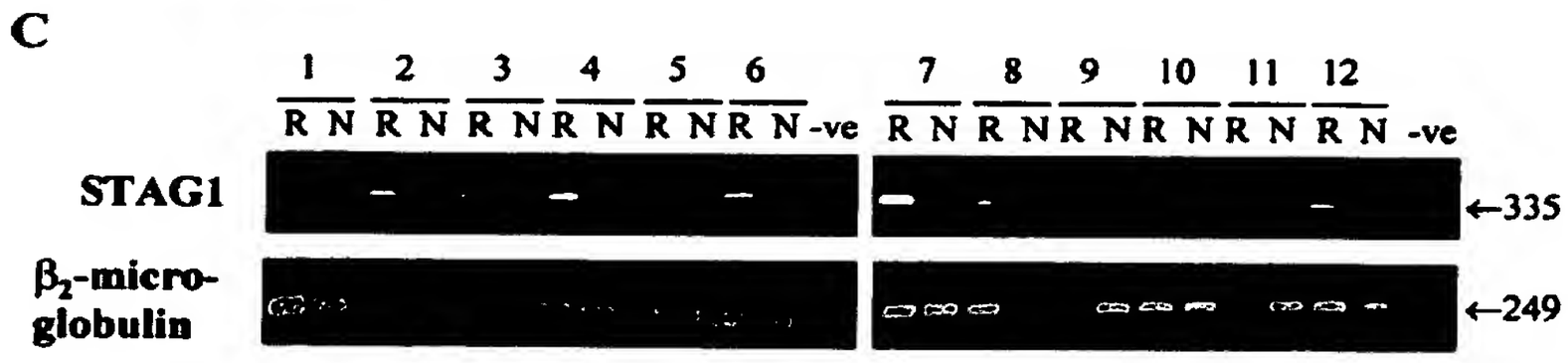
Signal intensity

C


1 2 3 4 5 6 7 8 9 10 11 12
R N R N R N R N R N R N -ve R N R N R N R N R N R N -ve
STAG1
β2-micro-globulin
←335
←249

lymphoma samples and hence was termed *STAG1* (solid tumor – associated gene 1). The full-length cDNA of *STAG1* encompassed the recently described androgen-regulated *PMEPA1* [10], and so we termed this gene *STAG1/PMEPA1*. *STAG1/PMEPA1* encoded a putative transmembrane protein containing potential binding sites for proteins with SH3 and WW domains.

The coding region of *STAG1/PMEPA1* showed 77% homology to another gene, *C18orf1*, which is located on chromosome 18 and encodes several transcript variants predominantly expressed in the brain [15]. Like *STAG1/PMEPA1*, *C18orf1* has a large 3′ UTR of 7.1 kb that contains 10 potential polyadenylation sites, a number of which are used to generate multiple transcripts. The 3.6 kb 3′ UTR of *STAG1/PMEPA1* contained three consensus polyadenylation signals; only two *STAG1/PMEPA1* transcripts (2.7 and 5 kb) were detected on Northern blot analysis. The polyadenylation signal at 2463 bp most likely was used to generate the 2.7-kb transcript, though analysis of the EST database showed that ESTs were generated from both the polyadenylation signals at nucleotides 2463 and 2158. *STAG1/PMEPA1* also had alternately spliced first exons. Besides sequence similarity, *C18orf1* and *STAG1/PMEPA1* both had a transmembrane domain, and the STAG1/PMEPA1 protein was of a size similar to the β1 isoform of *C18orf1*. These two genes most likely belong to a novel gene family whose functions remain to be elucidated.

The *STAG1/PMEPA1* cDNA contained three closely spaced potential translation-initiating codons at nucleotides 321–323, 333–335, and 420–422. The ATG that conformed most closely to a Kozak consensus motif (A/G)CCATGG) was that at 420–422 (AGCATGG). Our in vitro transcription/translation results indicated that STAG1/PMEPA1 protein synthesis could be initiated in vitro from the first or second ATG as well as from the third ATG. Use of the second or third ATG would result in truncation of the putative extracellular domain of STAG1/PMEPA1 by four and 33 amino acids, respectively. It will be important to determine which of these potential translation initiation sites is functional in vivo and the effect of protein truncation on STAG1/PMEPA1 protein function. In addition, the molecular weights obtained in vitro for STAG1/PMEPA1 of 36 kDa and 39 kDa were higher than the predicted molecular weights of 28 kDa and 32 kDa, respectively, indicating posttranslational modification of the protein. In support of this theory, there were a number of consensus sequences for posttranslational modifications, including glycosylation and *N*-myristoylation sites.

Although the role of STAG1/PMEPA1 is yet to be elucidated, the protein contained a putative transmembrane domain and motifs that suggest that it may bind SH3- and WW domain – containing proteins. SH3 domains are often present in eukaryotic signal transducing and cytoskeletal proteins and mediate such functions as protein-protein interactions and cell compartmentalization. These domains bind proline-rich sequences containing the core motif PXXP, where X denotes any amino acid [18]. Although the WW domain resembles the SH3 domain functionally by displaying affinity toward proline-rich ligands, their structures are distinct. WW domains, found in proteins that participate in cell signaling and regulation, form a binding pocket for ligands containing PPXY or PPLP core motifs, which usually are flanked by additional prolines [18]. The PPXY motif has been observed in a number of transcription factors, where it may play a role in transcriptional activation. Accordingly, STAG1/PMEPA1 may function by aggregating signaling molecules localizing the protein complex on the inner surface of a cellular membrane. Our analysis indicated that C18orf1 also contains potential SH3 and WW domain binding motifs.

Northern analysis and semiquantitative RT-PCR of RCC and normal kidney paired samples confirmed the upregulation of *STAG1/PMEPA1* in RCC. In addition, on analysis of the EST clones corresponding to *STAG1/PMEPA1* (Unigene EST clusters Hs.83883 and Hs.4299, containing 92 and 114

Figure 3. (A) Northern blot analysis of *STAG1/PMEPA1* expression in 16 normal human tissues. Hybridization was performed with cRNA probes generated from (i) the *STAG1/PMEPA1* 5′ end (nucleotides 1–1403) and (ii) the *STAG1/PMEPA1* 3′ UTR (nucleotides 4429–4763). (iii) A β-actin control probe was used to verify equivalent loading of RNA in each lane. (B) Graphical representation of signal intensities after hybridization with a probe generated from nucleotides 1–1403 of *STAG1/PMEPA1* cDNA to a Clontech Multiple Tissue Expression array containing polyA+ RNA from 76 different human tissues and cell lines. Tissues arrayed: 1, whole brain; 2, cerebral cortex; 3, frontal lobe; 4, parietal lobe; 5, occipital lobe; 6, temporal lobe; 7, cerebral cortex; 8, pons; 9, cerebellum, left; 10, cerebellum, right; 11, corpus callosum; 12, amygdala; 13, caudate nucleus; 14, hippocampus; 15, medulla oblongata; 16, putamen; 17, substantia nigra; 18, accumbens nucleus; 19, thalamus; 20, pituitary gland; 21, spinal cord; 22, heart; 23, aorta; 24, atrium, left; 25, atrium, right; 26, ventrical, left; 27, ventrical, right; 28, interventricular septum; 29, apex of heart; 30, esophagus; 31, stomach; 32, duodenum; 33, jejunum; 34, ileum; 35, ilocecum; 36, appendix; 37, colon, ascending; 38, colon, transverse; 39, colon, descending; 40, rectum; 41, kidney; 42, skeletal muscle; 43, spleen; 44, thymus; 45, peripheral blood leukocyte; 46, lymph node; 47, bone marrow; 48, trachea; 49, lung; 50, placenta; 51, bladder; 52, uterus; 53, prostate; 54, testis; 55, ovary; 56, liver; 57, pancreas; 58, adrenal gland; 59, thyroid gland; 60, salivary gland; 61, mammary gland; 62, leukemia, HL-60; 63, HeLa S3; 64, leukemia, K-562; 65, leukemia, MOLT-4; 66, Burkitt's lymphoma, Raji; 67, Burkitt's lymphoma, Daudi; 68, colorectal adenocarcinoma, SW480; 69, lung carcinoma, A549; 70, fetal brain; 71, fetal heart; 72, fetal kidney; 73, fetal liver; 74, fetal spleen; 75, fetal thymus; 76, fetal lung. (C) Semiquantitative RT-PCR for *STAG1/PMEPA1*. RT-PCR was performed on six female (1–6) and six male (7–12) paired RCC (R) and normal kidney (N) samples. Product sizes are indicated at the right. β_2-microglobulin was used as a control for cDNA synthesis, and no template PCR was performed as a negative control (–ve).

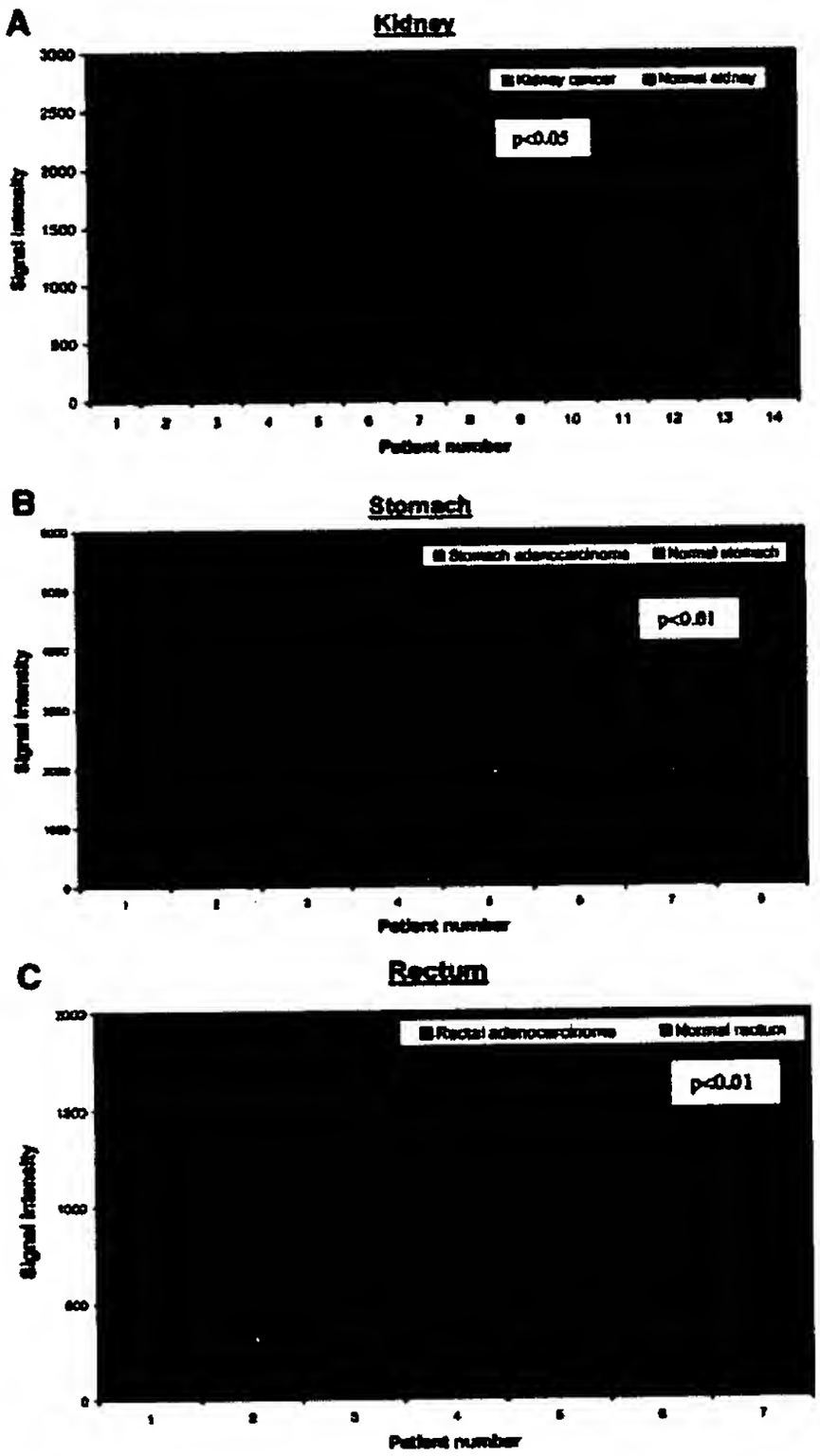


Figure 4. Graphical representation of signals obtained by probing a Matched Tumor/Normal Expression array containing cDNA from tumor and corresponding normal tissues from individual patients with a variety of cancers. Those tissues showing differential expression (kidney, RCC, stomach, and rectum—Figure 4A–C, respectively) are represented. The numbers on the y-axis denote arbitrary units obtained from densitometry analysis of the Matched Tumor/Normal array using ImageQuant software. Statistical analysis of cancer and normal samples for each tissue was performed using the Student's *t* test to obtain a *P* value.

clones, respectively), approximately 50% of the clones were found to be derived from tumor tissues. This is a significantly higher number than the general representation of ESTs from tumor tissue in EST database ($P < 0.001$), which has been reported as only 12% [19]. ESTs found to match *STAG1/PMEPA1* were frequently from a pancreas adenocarcinoma library, followed by a stomach cancer library. Hybridization of *STAG1/PMEPA1* to a tumor/normal matched array showed significantly increased expression in many stomach, rectal, and kidney cancers. Although only a small sample number has been examined and there are varying levels of expression between individual patients, these preliminary results indicate that the association of *STAG1/PMEPA1* with tumorigenesis warrants further investigation. Recently, comparative genomic hybridization was used to screen 47 primary stomach cancers for changes in the number of copies of DNA sequences [20]. High-level amplification was noted at a number of sites, including 20q13, and the most frequent alteration was the gain of 20q in 55% of cases.

In normal tissues, *STAG1/PMEPA1* was expressed abundantly only in the prostate. In a recent report *STAG1/PMEPA1* was identified from the prostate cancer cell line LNCaP using serial analysis of gene expression for genes induced in response to synthetic androgen R1881 treatment [10]. The expression of *STAG1/PMEPA1* in LNCaP cells was induced by androgen in a time-specific and dose-specific manner. *STAG1/PMEPA1* also was shown to be overexpressed in three of four androgen-independent secondary prostate tumors derived from a xenograft model. The authors did not note any increase of *STAG1/PMEPA1* expression in primary prostate tumors compared with normal prostate. Together with our findings that *STAG1/PMEPA1* was overexpressed in a number of solid tumors originating from tissues not highly regulated by androgens and that *STAG/PMEPA1* was expressed in RCC tumors from both male and female patients, these findings suggest that this gene has other important tissue-specific regulatory mechanisms. The finding of alternate exon 1 sequences also suggests that tissue-specific regulatory sequences, which may play a role in this process, may be present in the promoter regions 5′ of these alternate exons.

In summary, we have characterized the *STAG1/PMEPA1* gene, which encoded a putative transmembrane protein containing potential binding sites for proteins with SH3 and WW domains. *STAG1/PMEPA1* was highly expressed in normal prostate, and its expression also was upregulated in solid tumors of the kidney, stomach, and rectum. The functional role of this gene in the normal prostate and in tumorigenesis warrants further investigation.

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